Fig.1A.

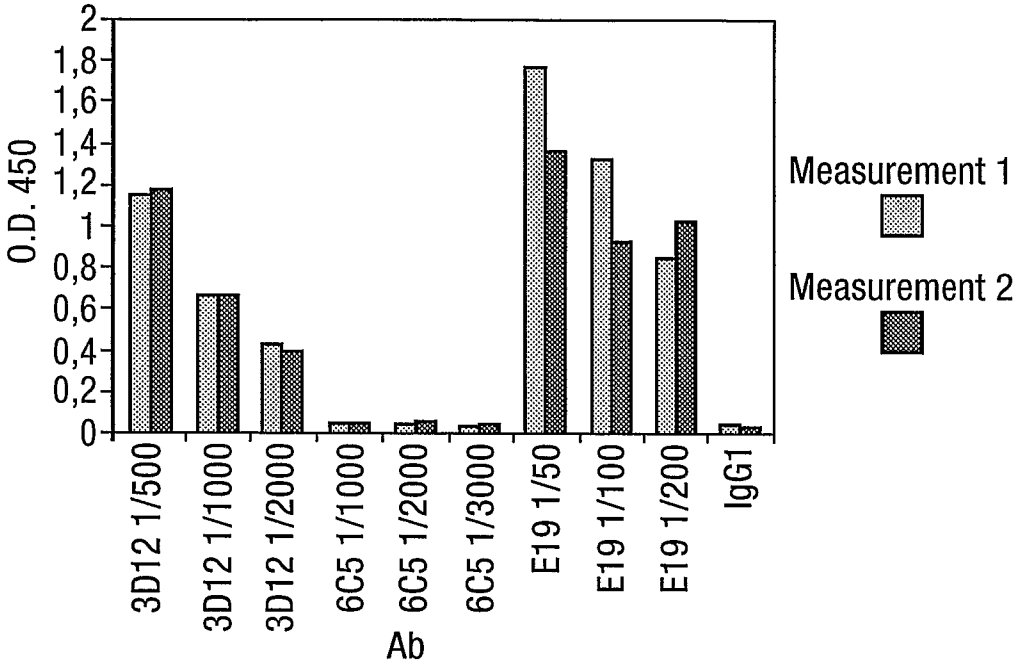

O.D. 450
2
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
3D12 1/500
3D12 1/1000
3D12 1/2000
6C5 1/1000
6C5 1/2000
6C5 1/3000
E19 1/50
E19 1/100
E19 1/200
IgG1
Ab
Measurement 1
Measurement 2

Fig.1B.

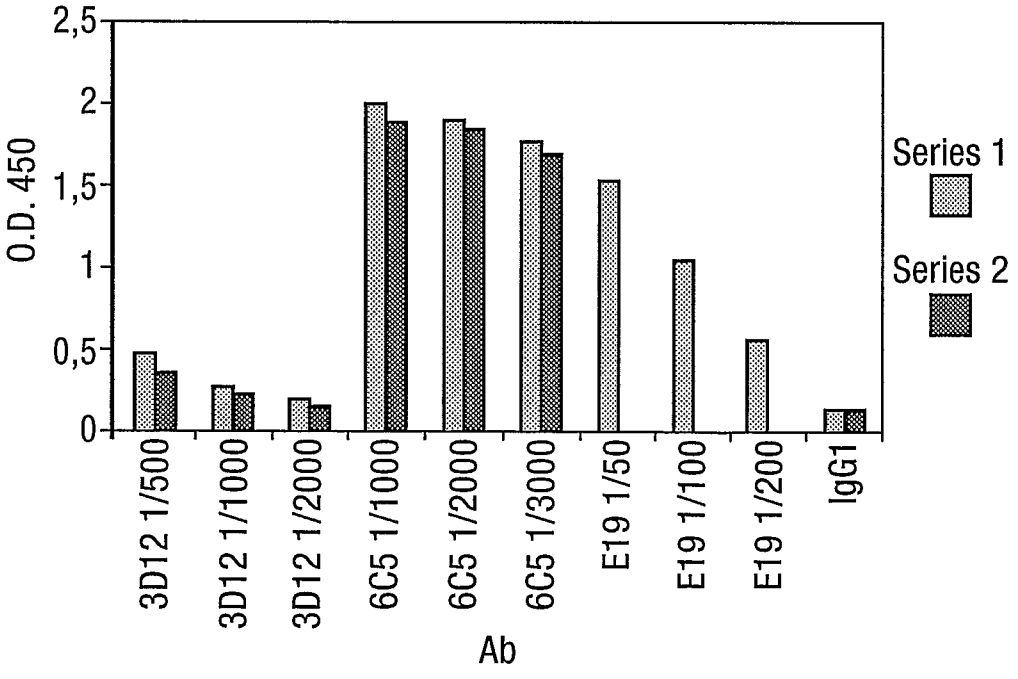
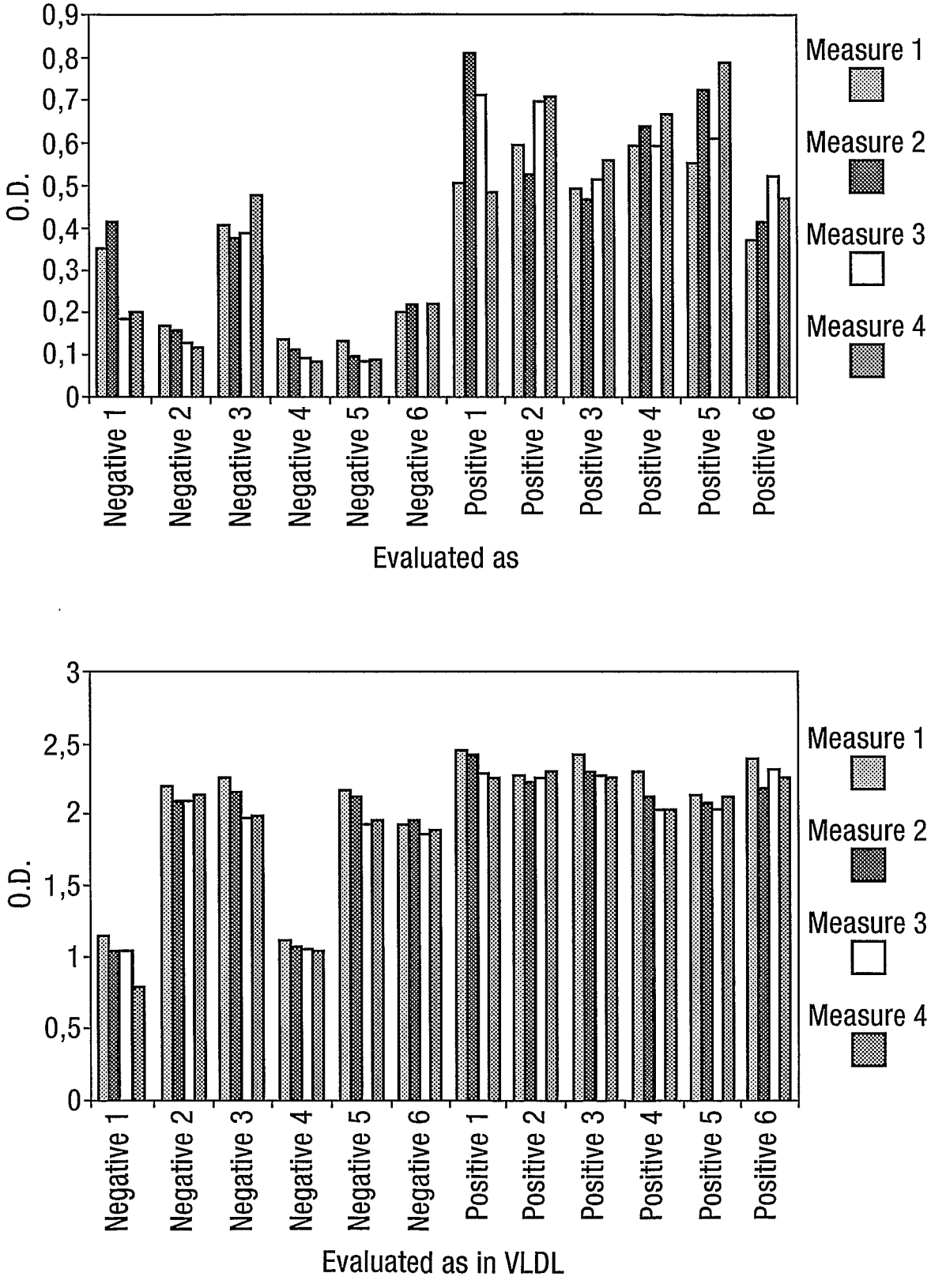

O.D. 450
2,5
2
1,5
1
0,5
0
3D12 1/500
3D12 1/1000
3D12 1/2000
6C5 1/1000
6C5 1/2000
6C5 1/3000
E19 1/50
E19 1/100
E19 1/200
IgG1
Ab
Series 1
Series 2

Fig.2.


0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
O.D.
Measure 1
Measure 2
Measure 3
Measure 4
Negative 1
Negative 2
Negative 3
Negative 4
Negative 5
Negative 6
Positive 1
Positive 2
Positive 3
Positive 4
Positive 5
Positive 6
Evaluated as
3
2,5
2
1,5
1
0,5
0
O.D.
Measure 1
Measure 2
Measure 3
Measure 4
Negative 1
Negative 2
Negative 3
Negative 4
Negative 5
Negative 6
Positive 1
Positive 2
Positive 3
Positive 4
Positive 5
Positive 6
Evaluated as in VLDL

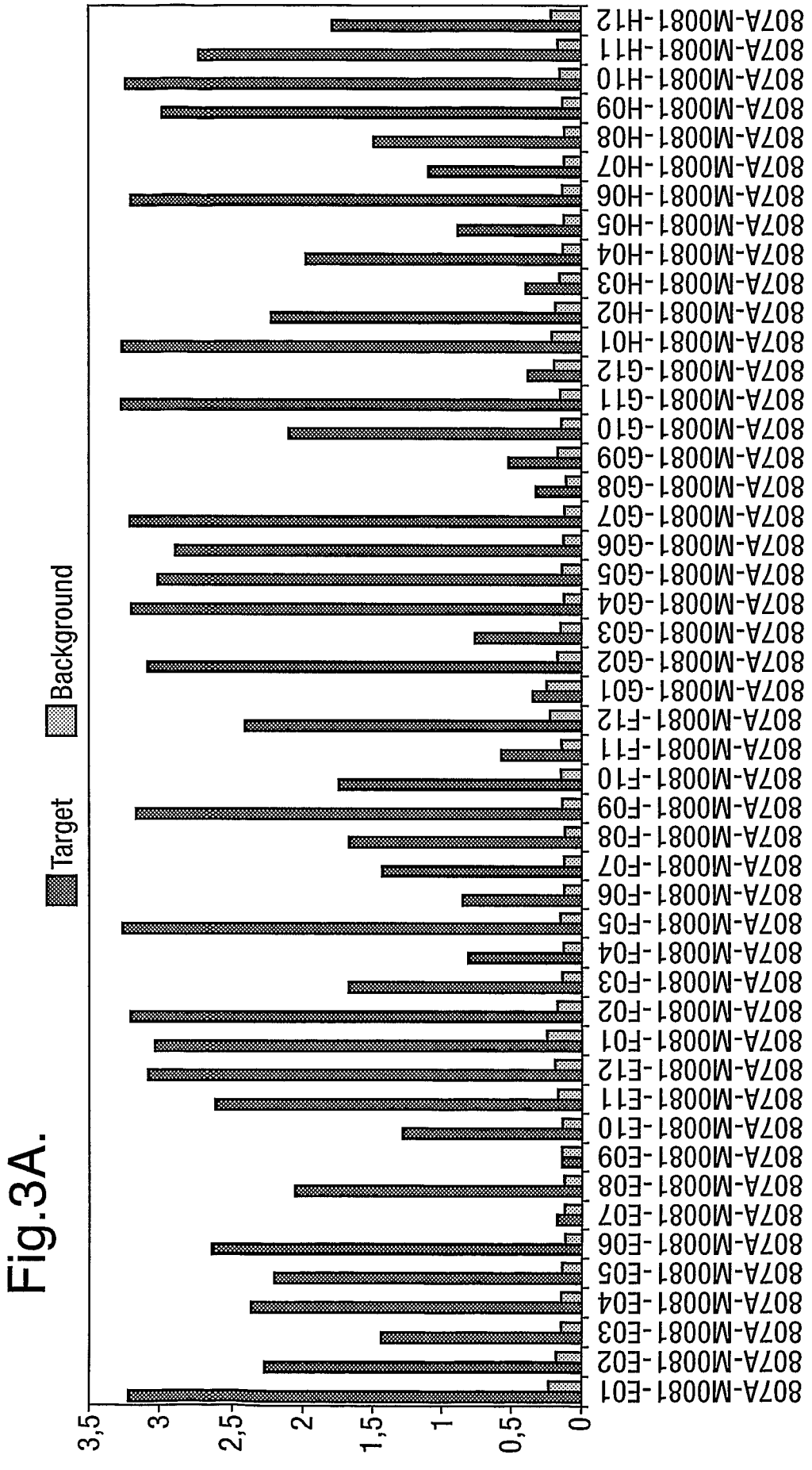

Target
Background
3,5
3
2,5
2
1,5
1
0,5
0
807A-M0081-E01
807A-M0081-E02
807A-M0081-E03
807A-M0081-E04
807A-M0081-E05
807A-M0081-E06
807A-M0081-E07
807A-M0081-E08
807A-M0081-E09
807A-M0081-E10
807A-M0081-E11
807A-M0081-E12
807A-M0081-F01
807A-M0081-F02
807A-M0081-F03
807A-M0081-F04
807A-M0081-F05
807A-M0081-F06
807A-M0081-F07
807A-M0081-F08
807A-M0081-F09
807A-M0081-F10
807A-M0081-F11
807A-M0081-F12
807A-M0081-G01
807A-M0081-G02
807A-M0081-G03
807A-M0081-G04
807A-M0081-G05
807A-M0081-G06
807A-M0081-G07
807A-M0081-G08
807A-M0081-G09
807A-M0081-G10
807A-M0081-G11
807A-M0081-G12
807A-M0081-H01
807A-M0081-H02
807A-M0081-H03
807A-M0081-H04
807A-M0081-H05
807A-M0081-H06
807A-M0081-H07
807A-M0081-H08
807A-M0081-H09
807A-M0081-H10
807A-M0081-H11
807A-M0081-H12

Fig.3A.

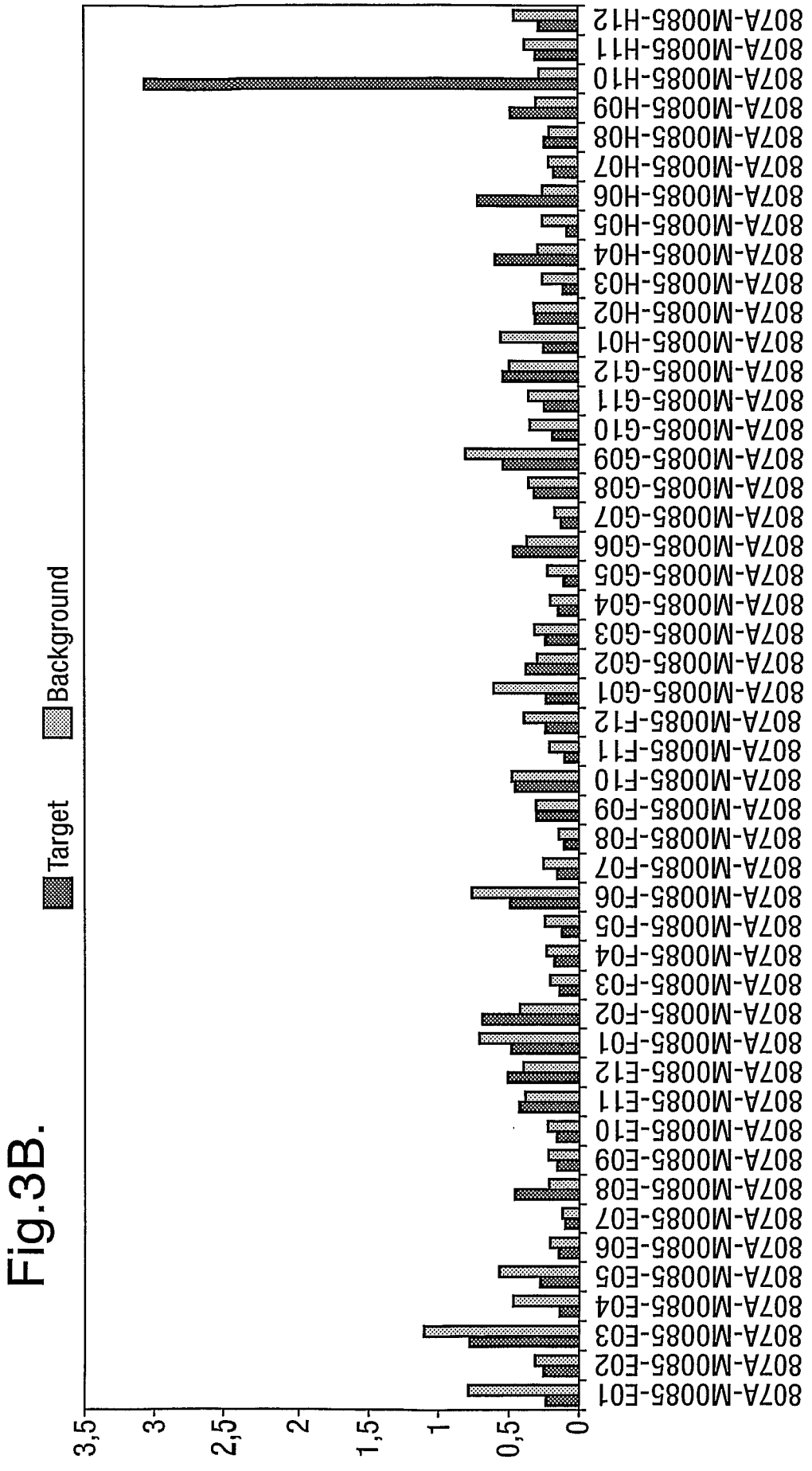

Target
Background
3,5
3
2,5
2
1,5
1
0,5
0
807A-M0085-E01
807A-M0085-E02
807A-M0085-E03
807A-M0085-E04
807A-M0085-E05
807A-M0085-E06
807A-M0085-E07
807A-M0085-E08
807A-M0085-E09
807A-M0085-E10
807A-M0085-E11
807A-M0085-E12
807A-M0085-F01
807A-M0085-F02
807A-M0085-F03
807A-M0085-F04
807A-M0085-F05
807A-M0085-F06
807A-M0085-F07
807A-M0085-F08
807A-M0085-F09
807A-M0085-F10
807A-M0085-F11
807A-M0085-F12
807A-M0085-G01
807A-M0085-G02
807A-M0085-G03
807A-M0085-G04
807A-M0085-G05
807A-M0085-G06
807A-M0085-G07
807A-M0085-G08
807A-M0085-G09
807A-M0085-G10
807A-M0085-G11
807A-M0085-G12
807A-M0085-H01
807A-M0085-H02
807A-M0085-H03
807A-M0085-H04
807A-M0085-H05
807A-M0085-H06
807A-M0085-H07
807A-M0085-H08
807A-M0085-H09
807A-M0085-H10
807A-M0085-H11
807A-M0085-H12

Fig.3B.

Fig.4A.

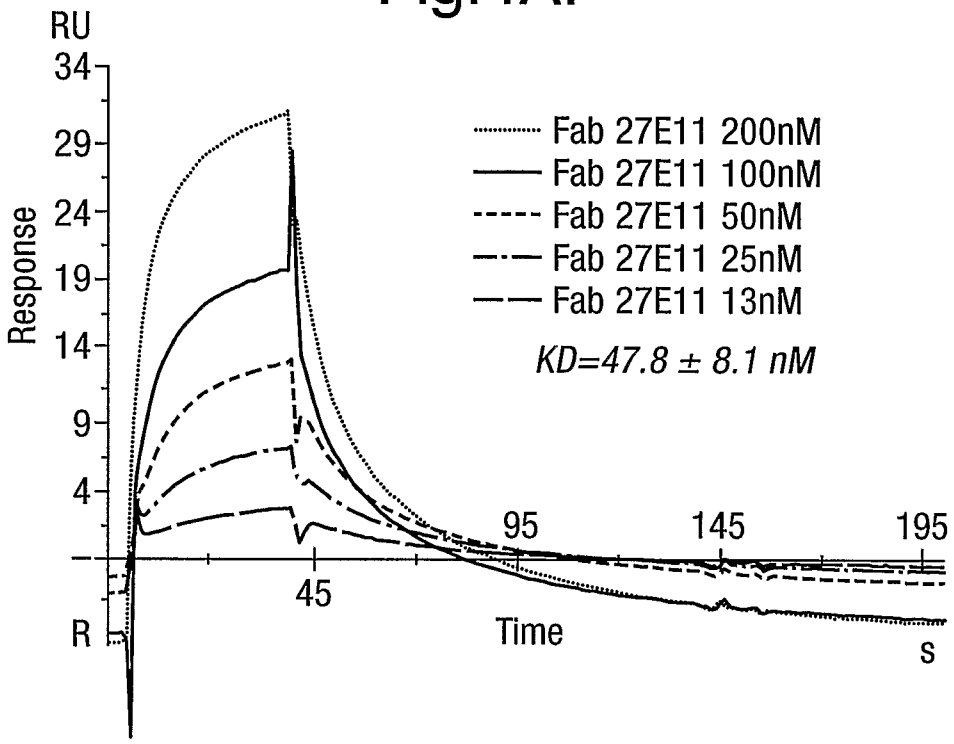

RU
34
29
24
19
14
9
4
R
Response
45
95
145
195
Time
s
Fab 27E11 200nM
Fab 27E11 100nM
Fab 27E11 50nM
Fab 27E11 25nM
Fab 27E11 13nM
KD=47.8 ± 8.1 nM

Fig.4B.

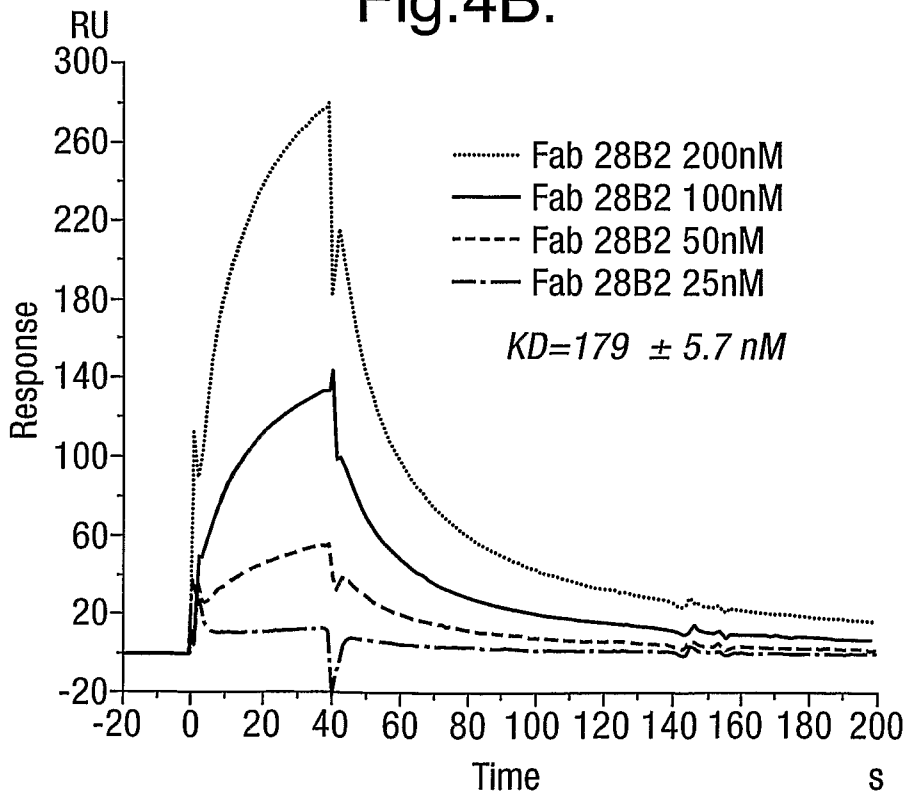

RU
300
260
220
180
140
100
60
20
-20
Response
-20 0 20 40 60 80 100 120 140 160 180 200
Time
s
Fab 28B2 200nM
Fab 28B2 100nM
Fab 28B2 50nM
Fab 28B2 25nM
KD=179 ± 5.7 nM

Fig.4C.

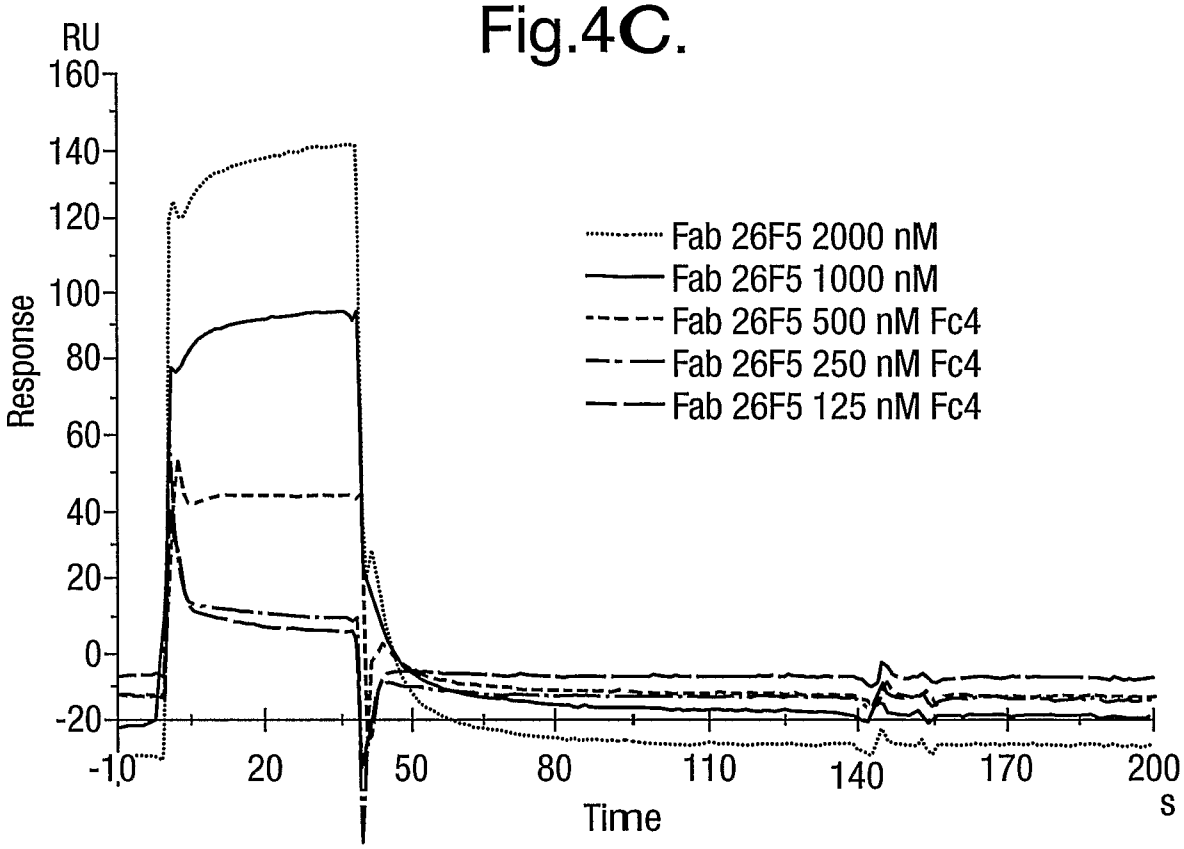

RU
160
140
120
100
80
60
40
20
0
-20
Response
-10
20
50
80
110
140
170
200
s
Time
Fab 26F5 2000 nM
Fab 26F5 1000 nM
Fab 26F5 500 nM Fc4
Fab 26F5 250 nM Fc4
Fab 26F5 125 nM Fc4

Fig.4D.

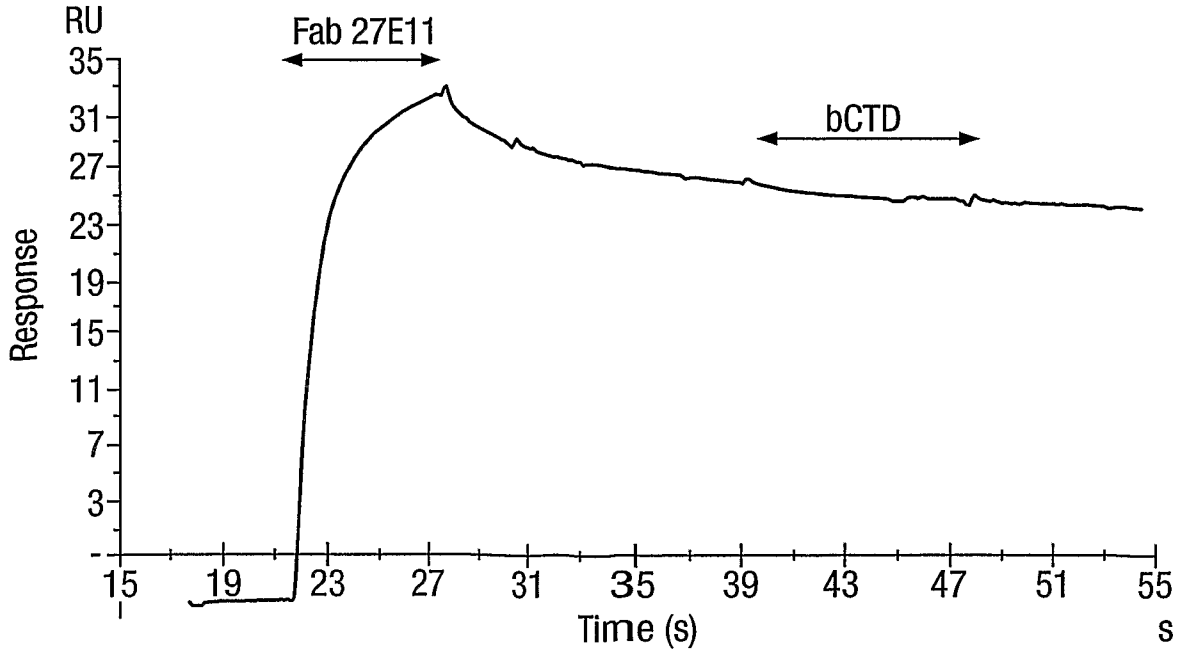
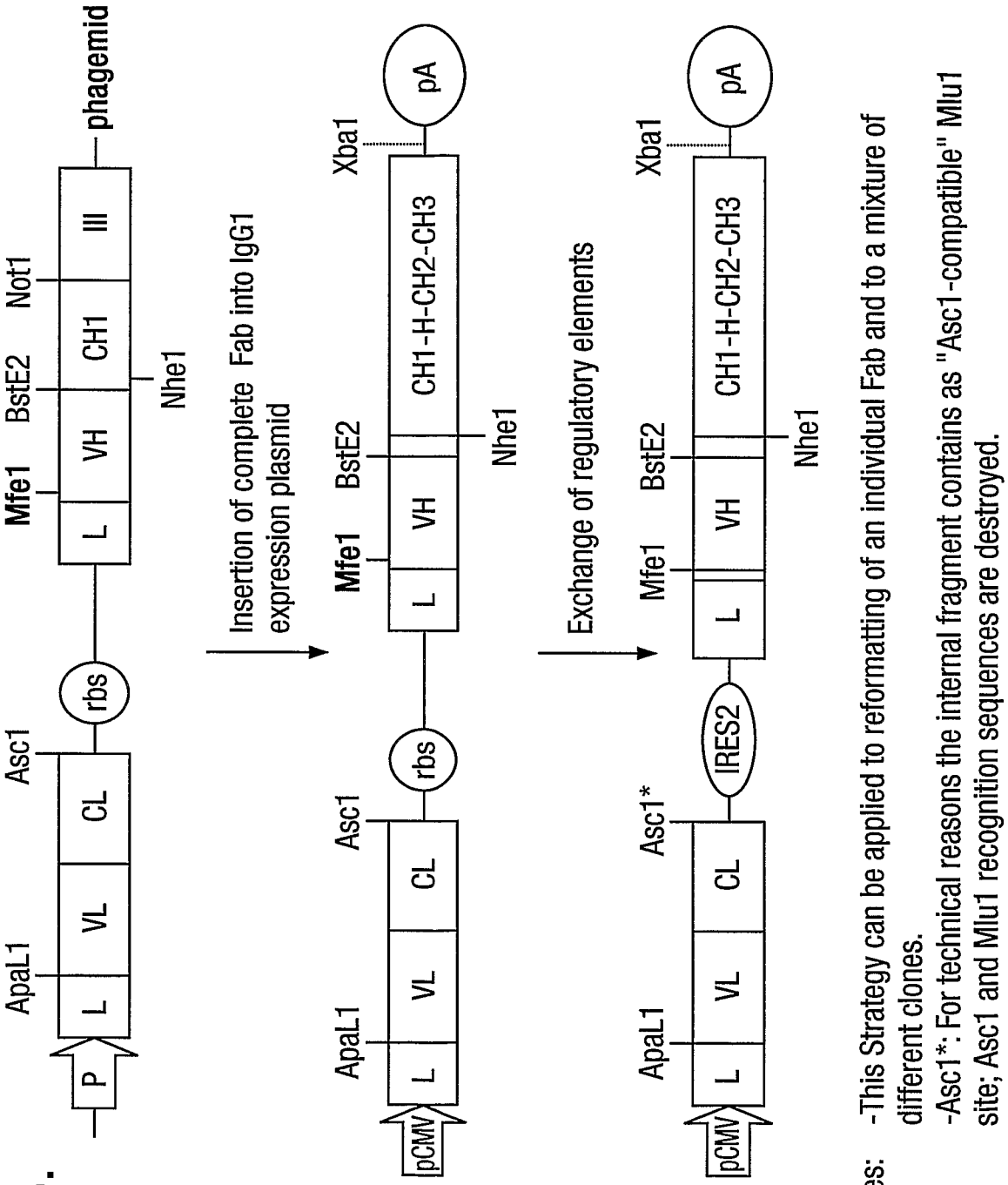

RU
35
31
27
23
19
15
11
7
3
-
Response
Fab 27E11
bCTD
15
19
23
27
31
35
39
43
47
51
55
Time (s)
s

Fig.5.



Notes: -This Strategy can be applied to reformatting of an individual Fab and to a mixture of different clones.

-Asc1*: For technical reasons the internal fragment contains as "Asc1-compatible" Mlu1 site; Asc1 and Mlu1 recognition sequences are destroyed.

Fig.6A.

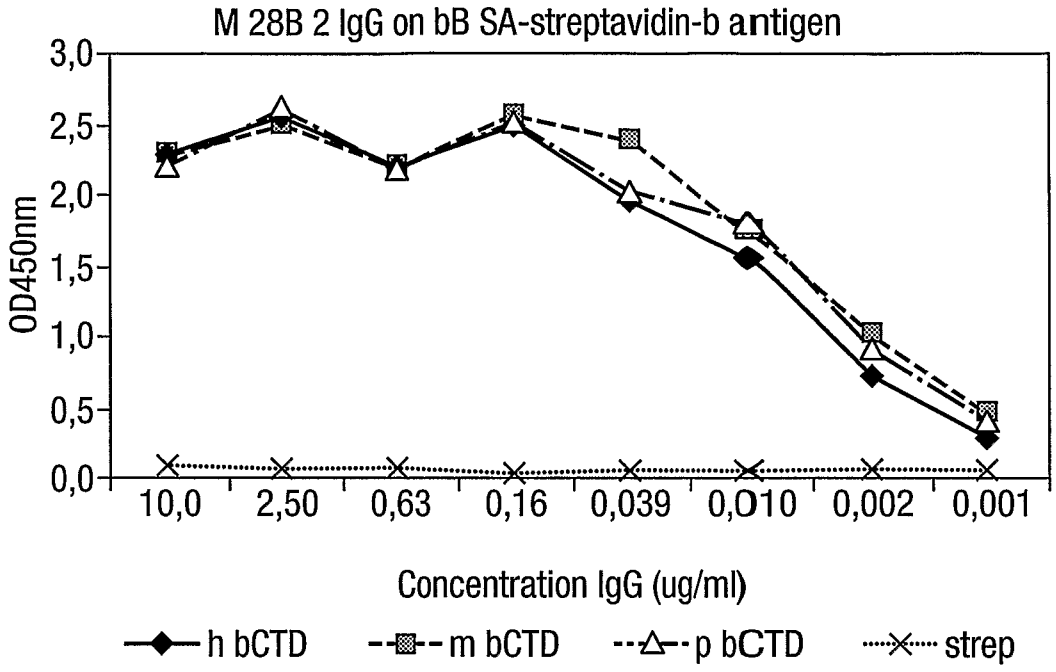

M 28B 2 IgG on bB SA-streptavidin-b antigen
OD450nm
3,0
2,5
2,0
1,5
1,0
0,5
0,0
10,0
2,50
0,63
0,16
0,039
0,010
0,002
0,001
Concentration IgG (ug/ml)
h bCTD
m bCTD
p bCTD
strep

Fig.6B.

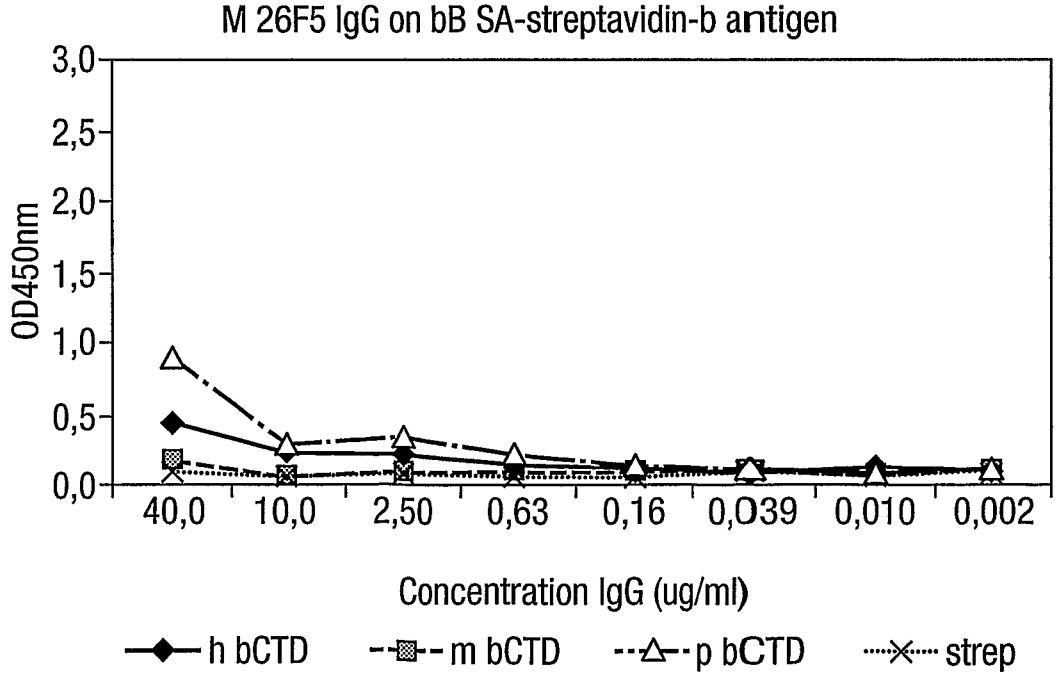

M 26F5 IgG on bB SA-streptavidin-b antigen
OD450nm
3,0
2,5
2,0
1,5
1,0
0,5
0,0
40,0
10,0
2,50
0,63
0,16
0,039
0,010
0,002
Concentration IgG (ug/ml)
h bCTD
m bCTD
p bCTD
strep

Fig.6C.

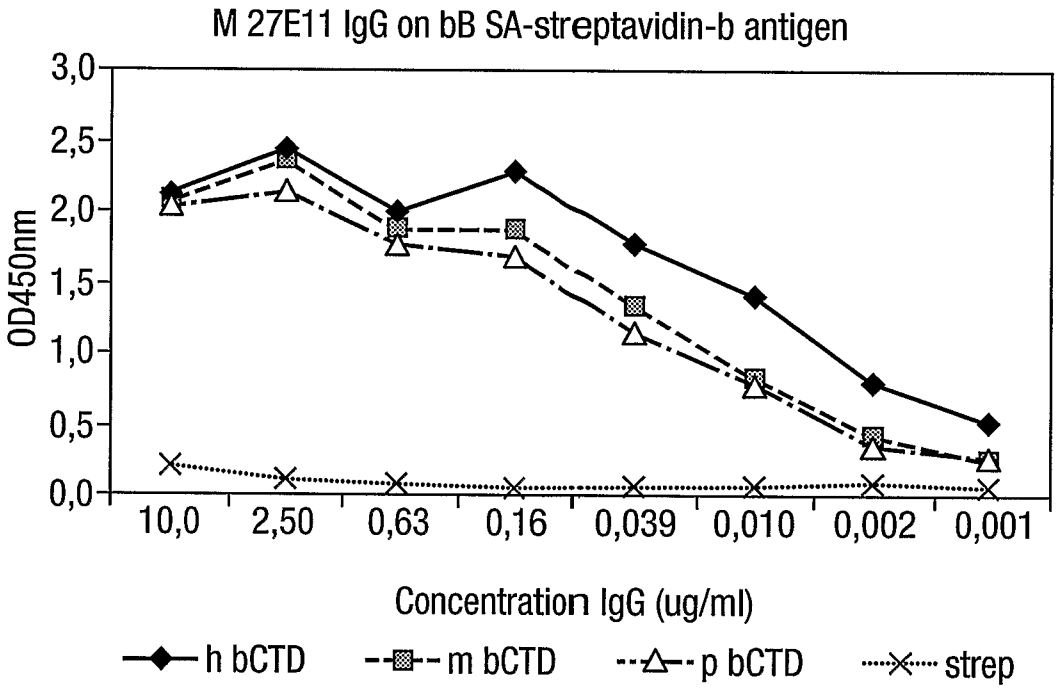

M 27E11 IgG on bB SA-streptavidin-b antigen
OD450nm
3,0
2,5
2,0
1,5
1,0
0,5
0,0
10,0
2,50
0,63
0,16
0,039
0,010
0,002
0,001
Concentration IgG (ug/ml)
h bCTD
m bCTD
p bCTD
strep

Fig.7A.

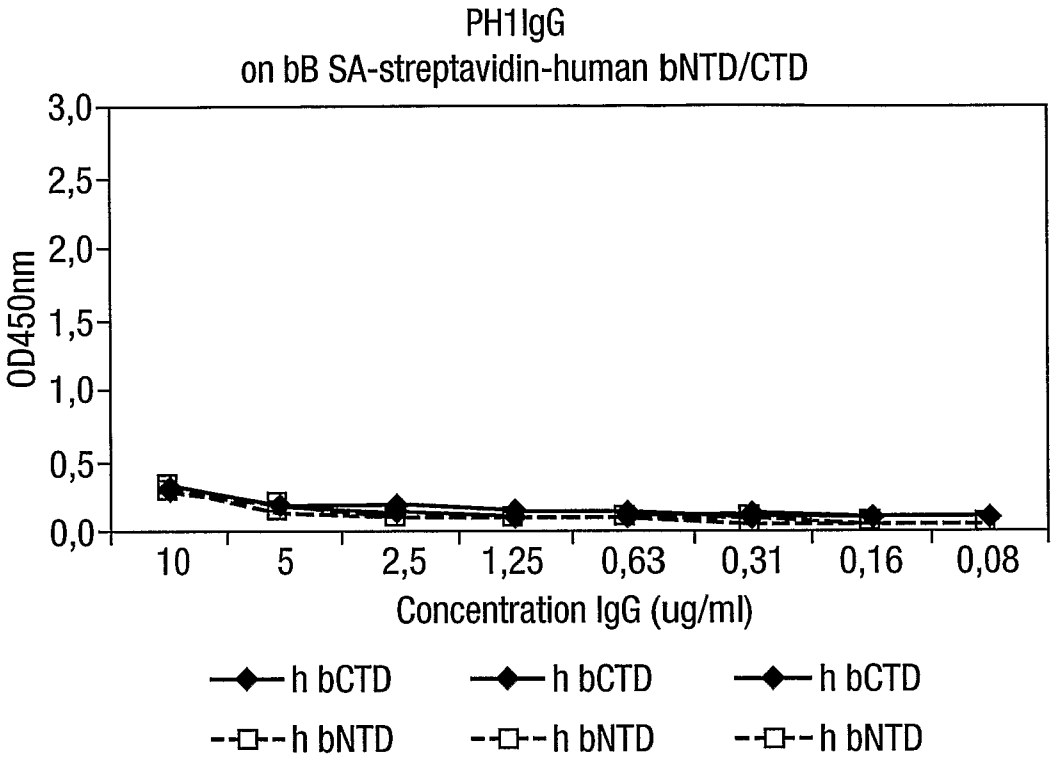

PH1IgG
on bB SA-streptavidin-human bNTD/CTD
OD450nm
3,0
2,5
2,0
1,5
1,0
0,5
0,0
10
5
2,5
1,25
0,63
0,31
0,16
0,08
Concentration IgG (ug/ml)
h bCTD
h bCTD
h bCTD
h bNTD
h bNTD
h bNTD

Fig.7B.

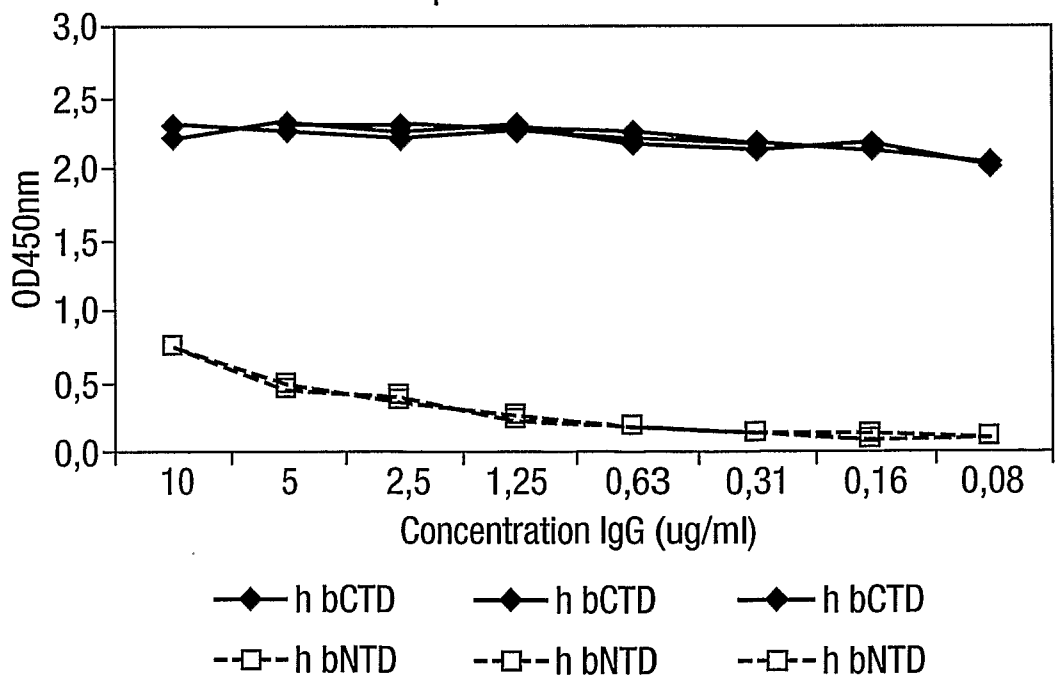

M 28B2 IgG
on bB SA-streptavidin-human bNTD/CTD
OD450nm
3,0
2,5
2,0
1,5
1,0
0,5
0,0
10
5
2,5
1,25
0,63
0,31
0,16
0,08
Concentration IgG (ug/ml)
h bCTD
h bCTD
h bCTD
h bNTD
h bNTD
h bNTD

Fig.8A.

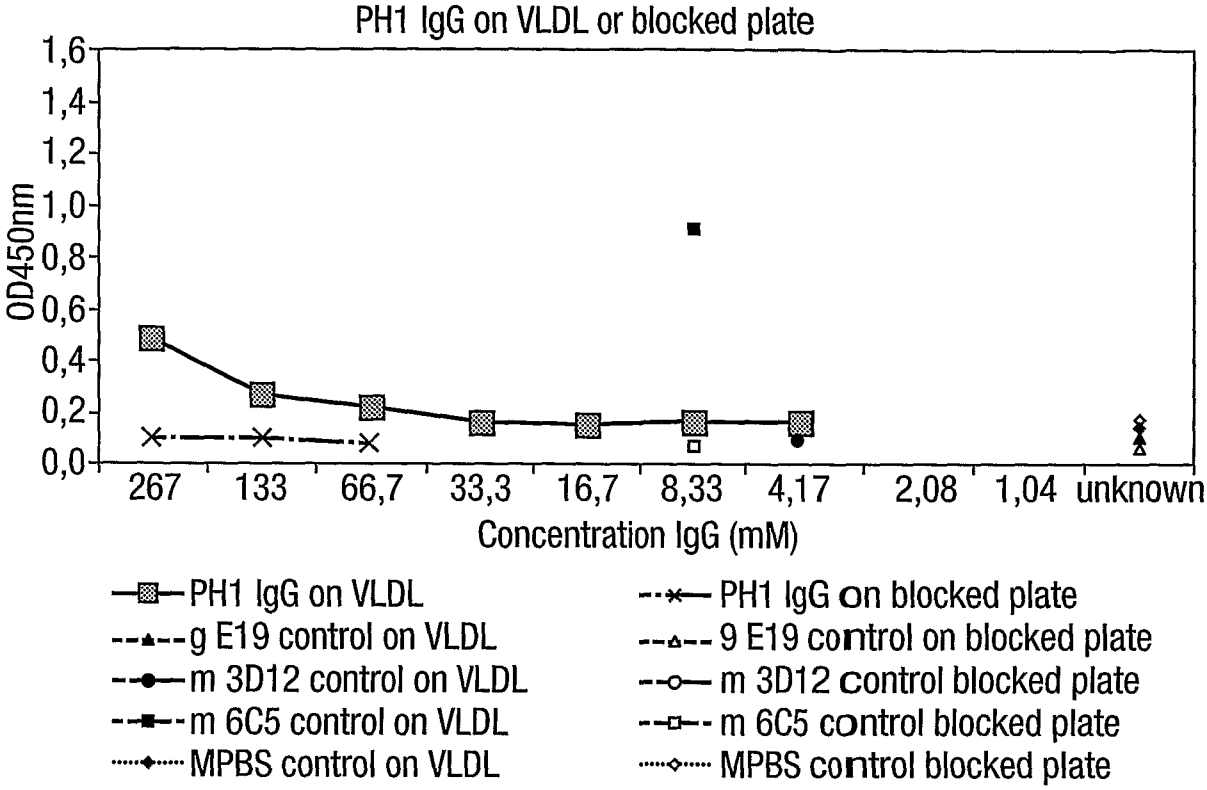

PH1 IgG on VLDL or blocked plate
OD450nm
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
267
133
66,7
33,3
16,7
8,33
4,17
2,08
1,04
unknown
Concentration IgG (mM)
PH1 IgG on VLDL
g E19 control on VLDL
m 3D12 control on VLDL
m 6C5 control on VLDL
MPBS control on VLDL
PH1 IgG on blocked plate
9 E19 control on blocked plate
m 3D12 control blocked plate
m 6C5 control blocked plate
MPBS control blocked plate

Fig.8B.

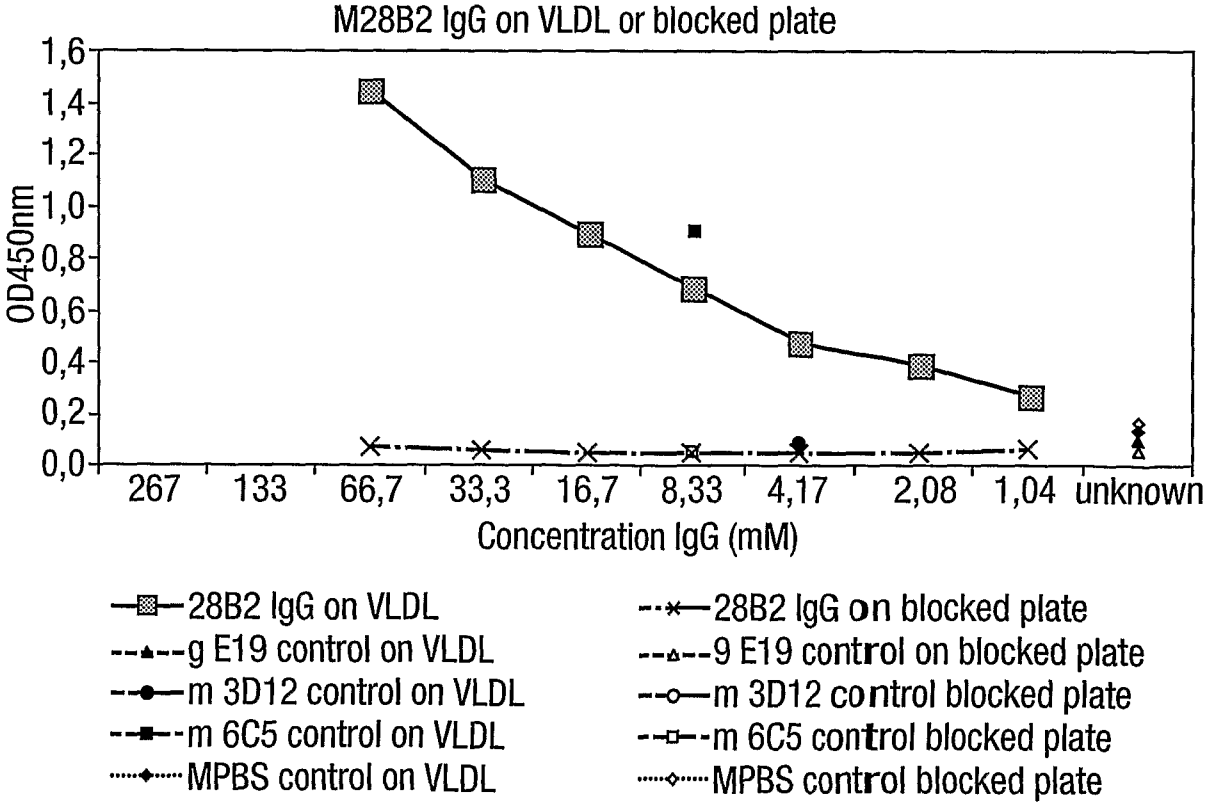

M28B2 IgG on VLDL or blocked plate
OD450nm
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
267
133
66,7
33,3
16,7
8,33
4,17
2,08
1,04
unknown
Concentration IgG (mM)
28B2 IgG on VLDL
g E19 control on VLDL
m 3D12 control on VLDL
m 6C5 control on VLDL
MPBS control on VLDL
28B2 IgG on blocked plate
9 E19 control on blocked plate
m 3D12 control blocked plate
m 6C5 control blocked plate
MPBS control blocked plate

Fig.9A.

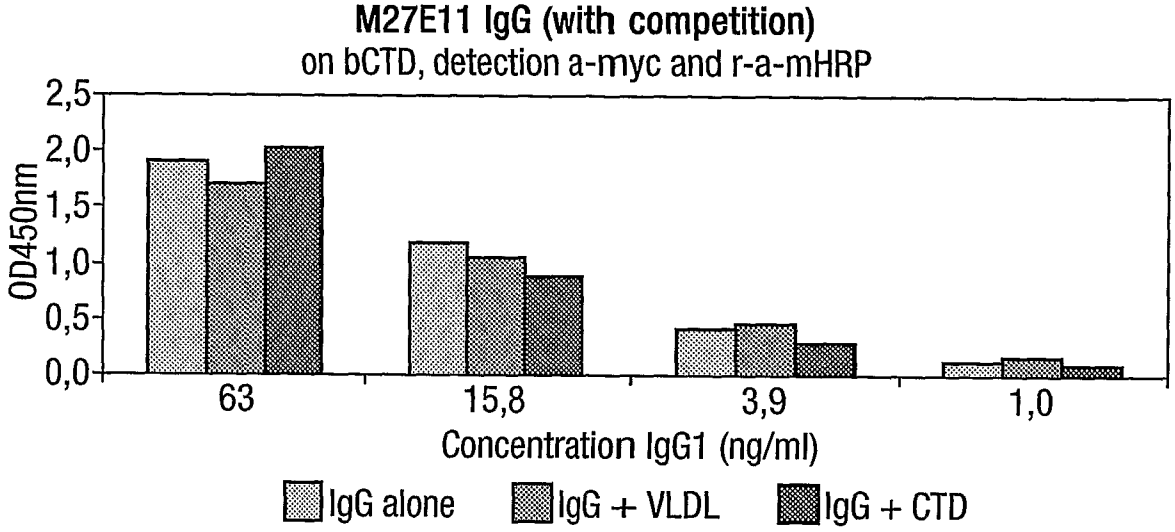

M27E11 IgG (with competition)
on bCTD, detection a-myc and r-a-mHRP
OD450nm
2,5
2,0
1,5
1,0
0,5
0,0
63
15,8
3,9
1,0
Concentration IgG1 (ng/ml)
IgG alone
IgG + VLDL
IgG + CTD

Fig.9B.

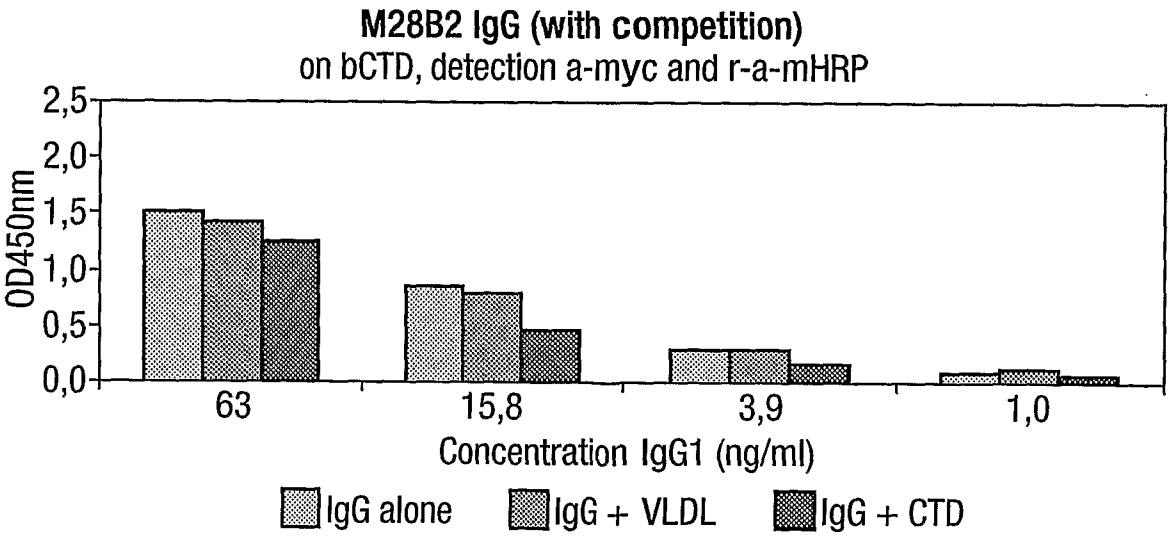

M28B2 IgG (with competition)
on bCTD, detection a-myc and r-a-mHRP
OD450nm
2,5
2,0
1,5
1,0
0,5
0,0
63
15,8
3,9
1,0
Concentration IgG1 (ng/ml)
IgG alone
IgG + VLDL
IgG + CTD

Fig.9C.

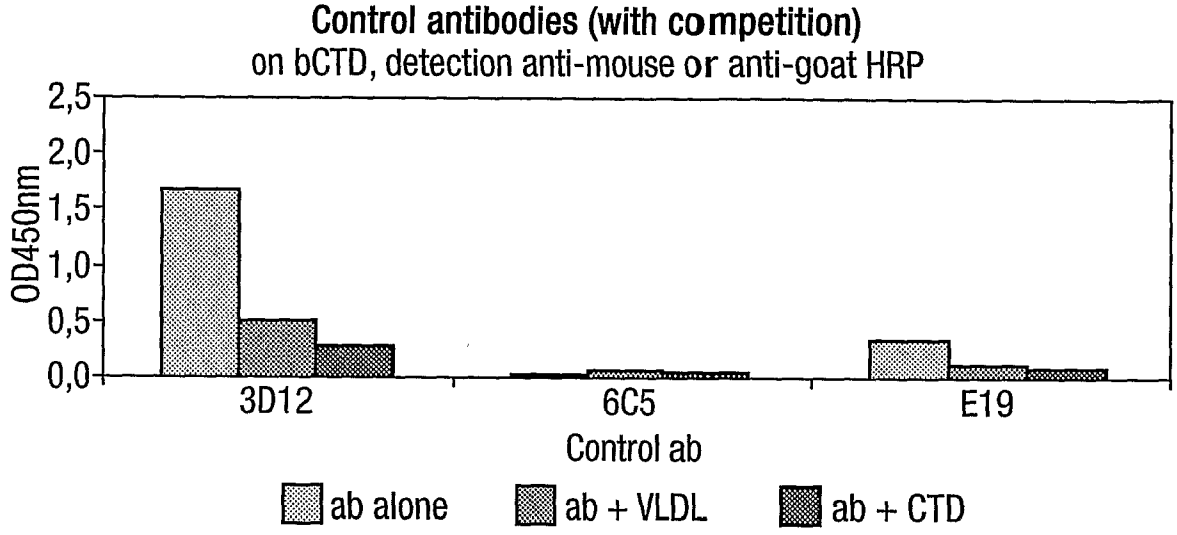

Control antibodies (with competition)
on bCTD, detection anti-mouse or anti-goat HRP
OD450nm
2,5
2,0
1,5
1,0
0,5
0,0
3D12
6C5
E19
Control ab
ab alone
ab + VLDL
ab + CTD

Fig.9D.

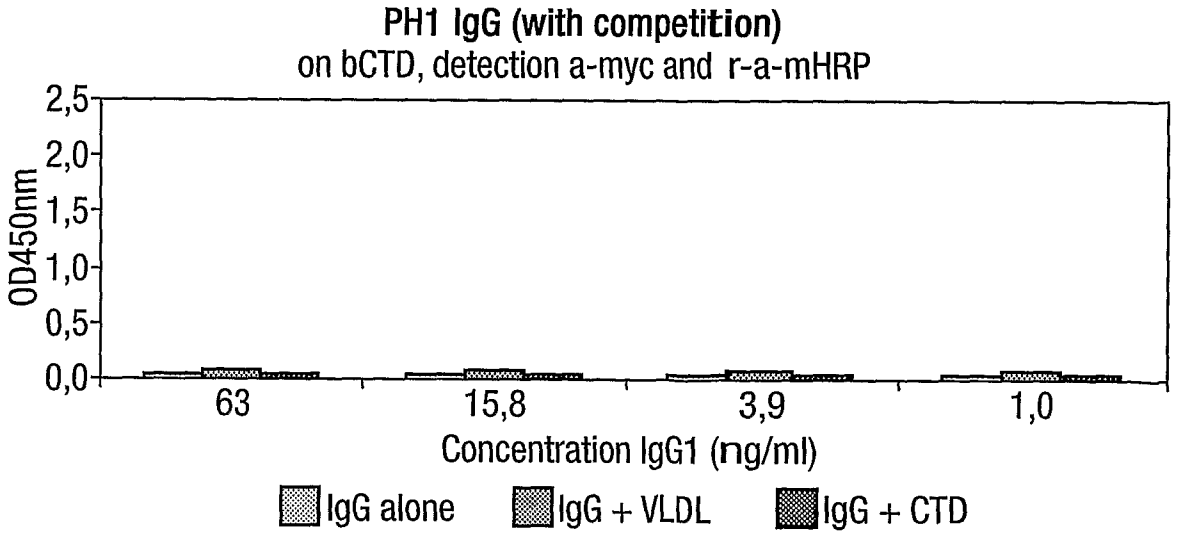

PH1 IgG (with competition)
on bCTD, detection a-myc and r-a-mHRP
OD450nm
2,5
2,0
1,5
1,0
0,5
0,0
63
15,8
3,9
1,0
Concentration IgG1 (ng/ml)
IgG alone
IgG + VLDL
IgG + CTD

Fig.10.

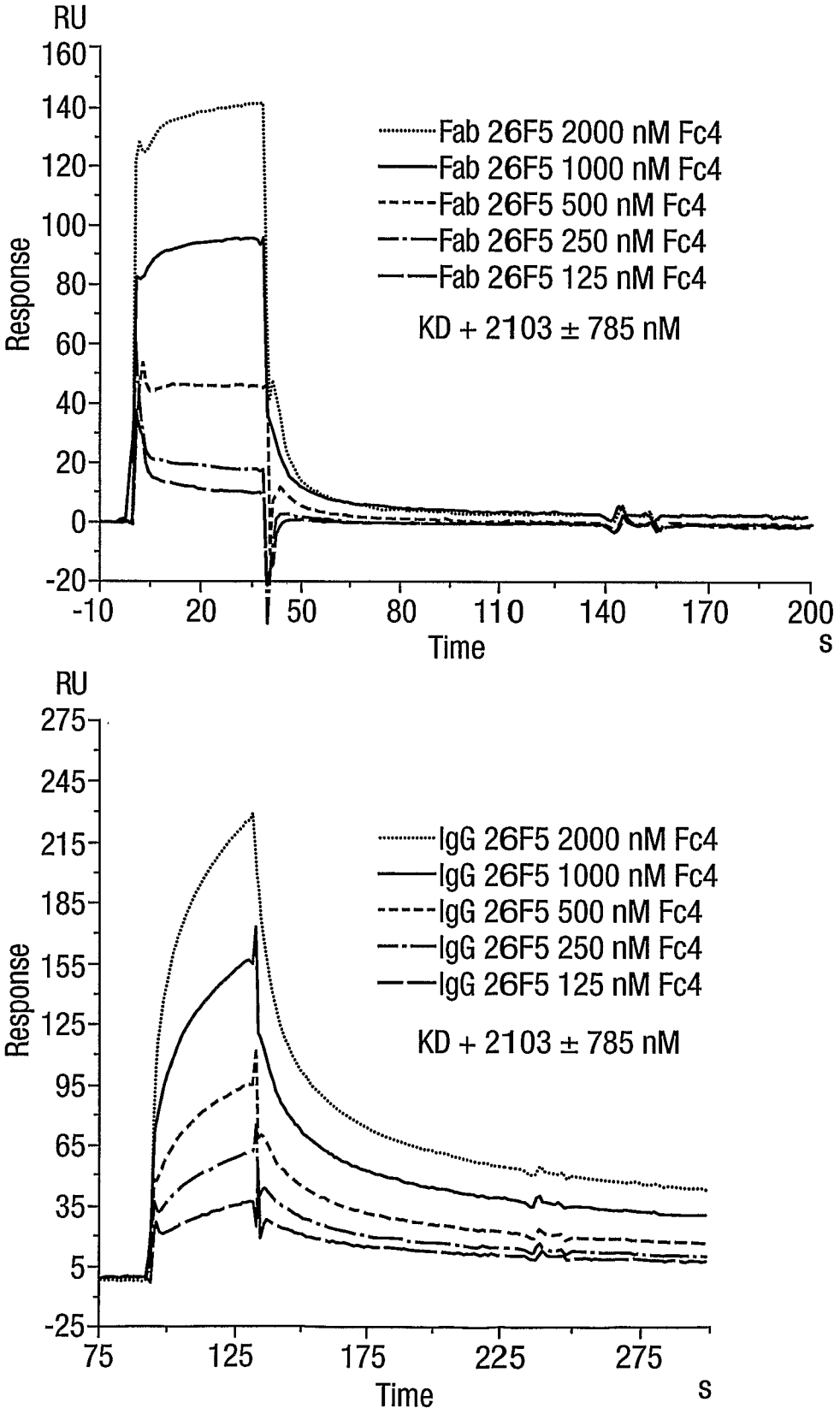
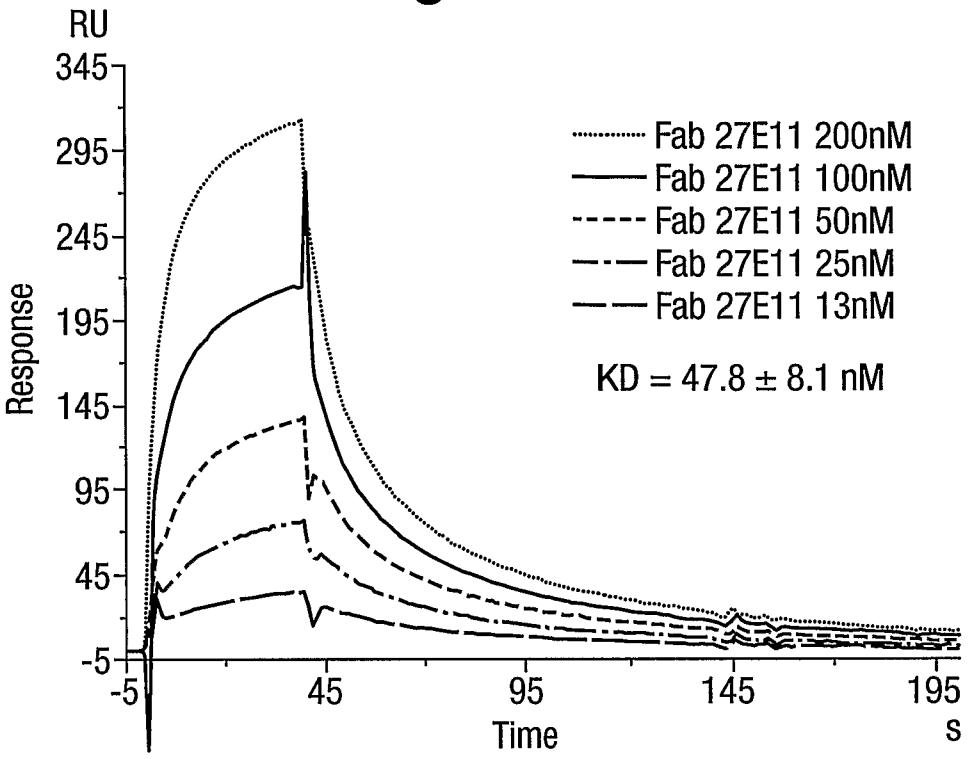

RU
160
140
120
100
80
60
40
20
0
-20
Response
-10
20
50
80
110
140
170
200
Time
s
Fab 26F5 2000 nM Fc4
Fab 26F5 1000 nM Fc4
Fab 26F5 500 nM Fc4
Fab 26F5 250 nM Fc4
Fab 26F5 125 nM Fc4
KD + 2103 ± 785 nM
RU
275
245
215
185
155
125
95
65
35
5
-25
Response
75
125
175
225
275
Time
s
IgG 26F5 2000 nM Fc4
IgG 26F5 1000 nM Fc4
IgG 26F5 500 nM Fc4
IgG 26F5 250 nM Fc4
IgG 26F5 125 nM Fc4
KD + 2103 ± 785 nM

Fig.11A.


RU
345
295
245
195
145
95
45
-5
Response
Fab 27E11 200nM
Fab 27E11 100nM
Fab 27E11 50nM
Fab 27E11 25nM
Fab 27E11 13nM
KD = 47.8 ± 8.1 nM
-5
45
95
145
195
Time
s

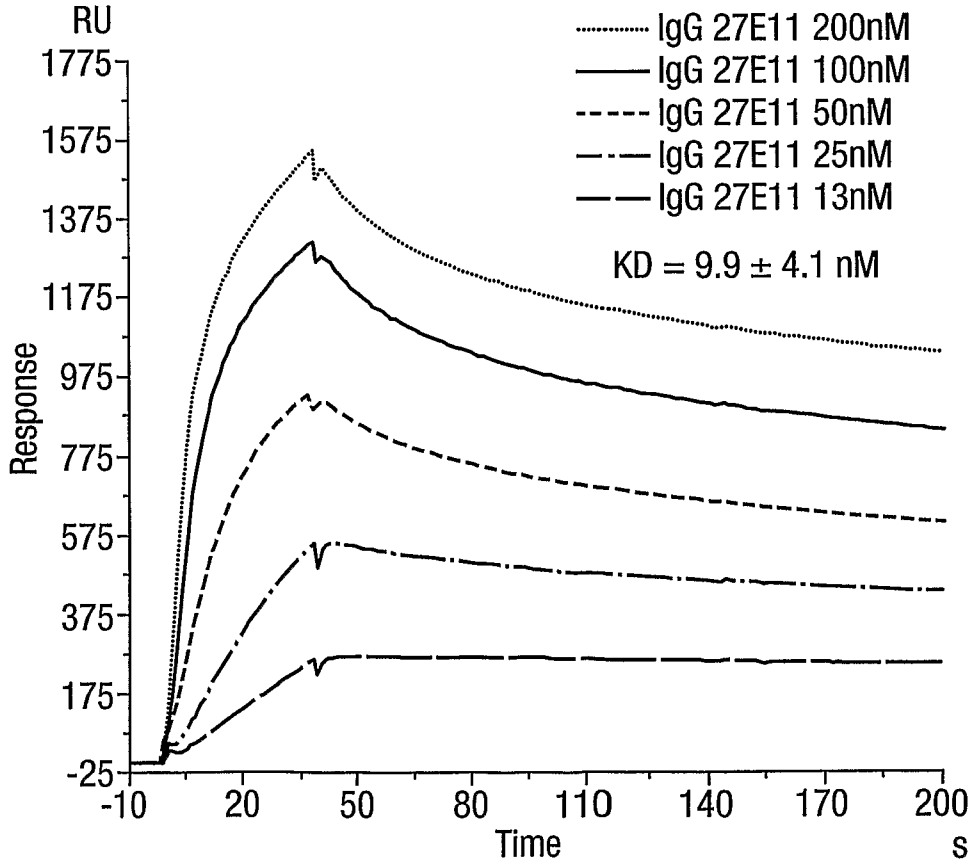
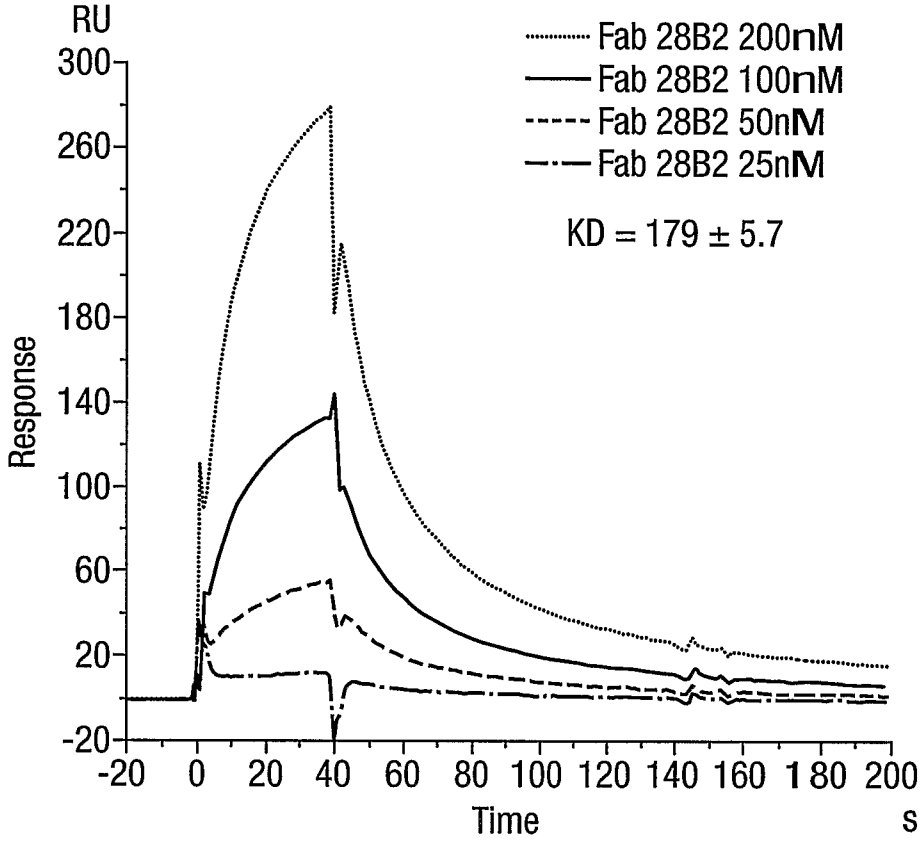

RU
1775
1575
1375
1175
975
775
575
375
175
-25
Response
IgG 27E11 200nM
IgG 27E11 100nM
IgG 27E11 50nM
IgG 27E11 25nM
IgG 27E11 13nM
KD = 9.9 ± 4.1 nM
-10
20
50
80
110
140
170
200
Time
s

Fig.11B.


RU
300
260
220
180
140
100
60
20
-20
Response
Fab 28B2 200nM
Fab 28B2 100nM
Fab 28B2 50nM
Fab 28B2 25nM
KD = 179 ± 5.7
-20 0 20 40 60 80 100 120 140 160 180 200
Time
s

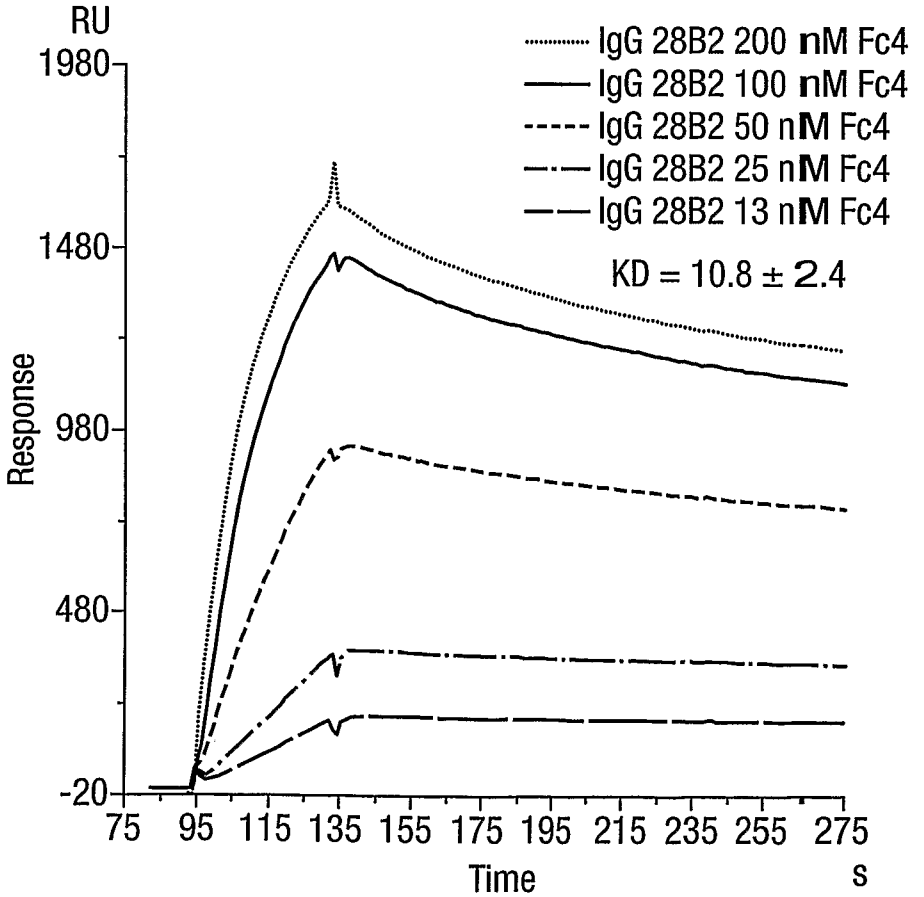

RU
1980
1480
980
480
-20
Response
IgG 28B2 200 nM Fc4
IgG 28B2 100 nM Fc4
IgG 28B2 50 nM Fc4
IgG 28B2 25 nM Fc4
IgG 28B2 13 nM Fc4
KD = 10.8 ± 2.4
75 95 115 135 155 175 195 215 235 255 275
Time
s

Fig.12.

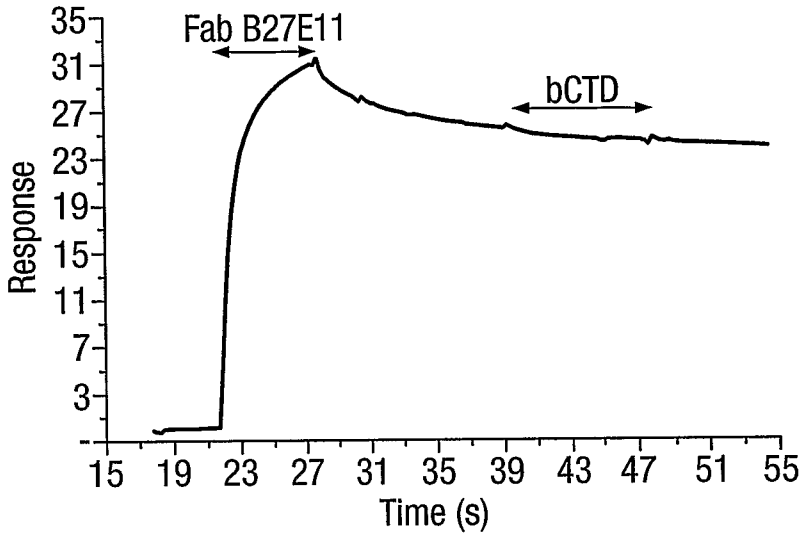

Fab B27E11
bCTD
Response
35
31
27
23
19
15
11
7
3
15 19 23 27 31 35 39 43 47 51 55
Time (s)

Fig.13.

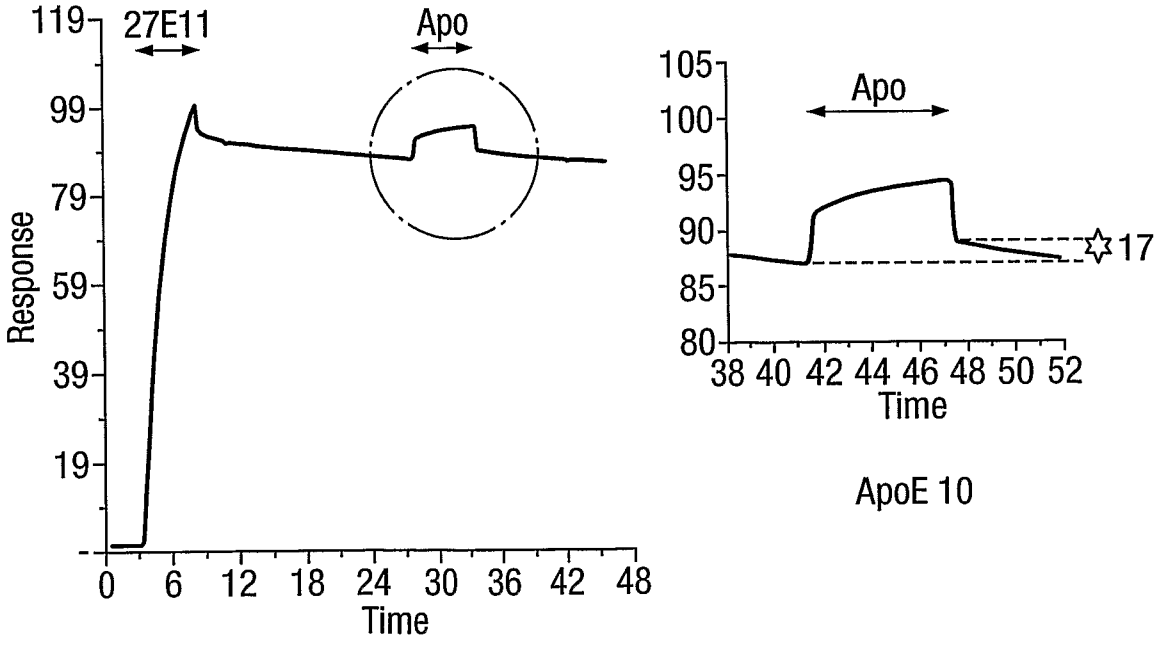

27E11
Apo
Response
119
99
79
59
39
19
0 6 12 18 24 30 36 42 48
Time
Apo
105
100
95
90
85
80
17
38 40 42 44 46 48 50 52
Time
ApoE 10

Fig.14.

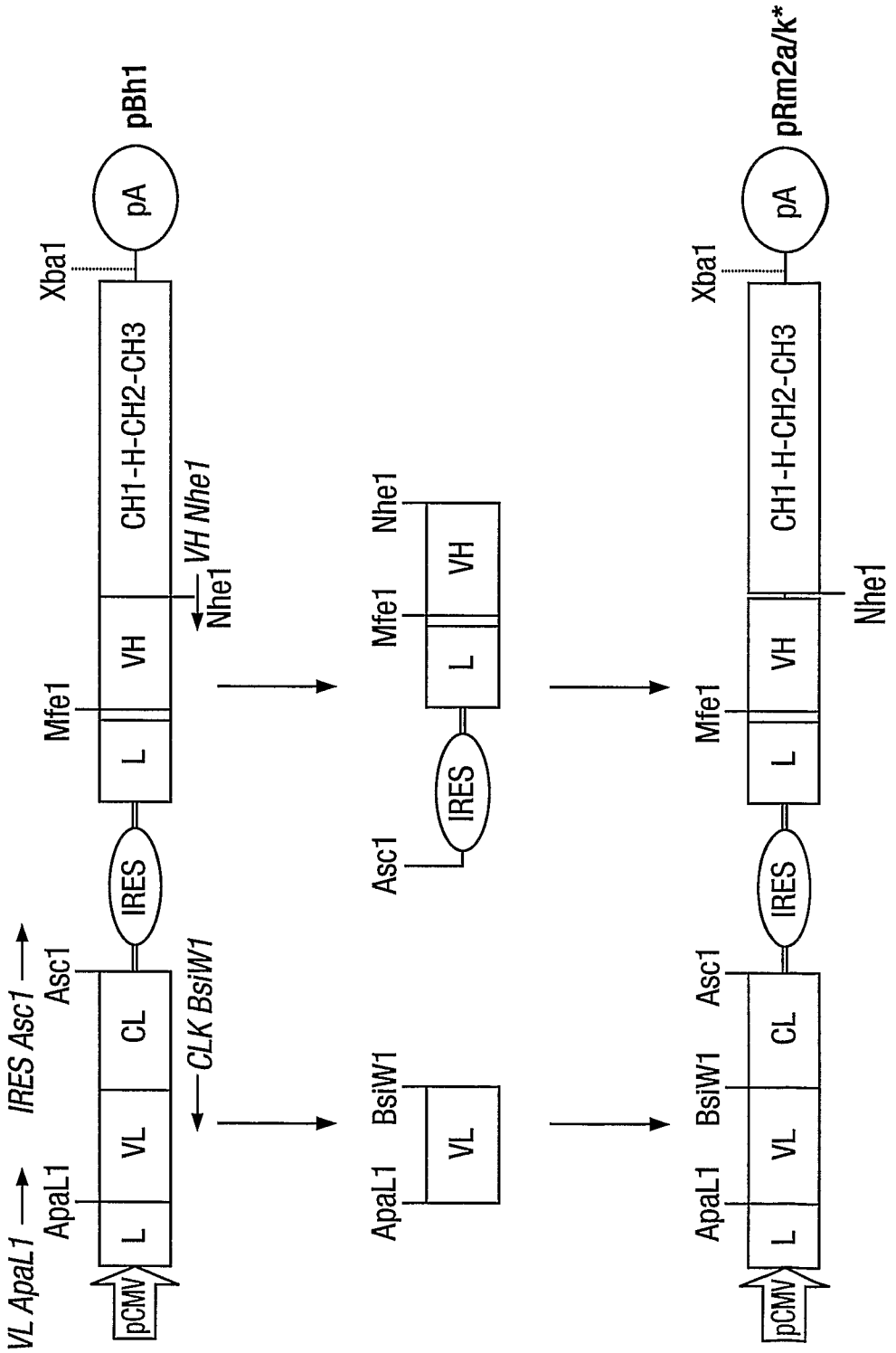

pBh1
pA
Xba1
CH1-H-CH2-CH3
VH Nhe1
Nhe1
Mfe1
VH
L
IRES
IRES Asc1
Asc1
CL
CLK BsiW1
VL
VL ApaL1
ApaL1
L
pCMV
pRm2a/k*

Fig.15.

```
peptide 1: biotin-GC-ARMEEMGSRTRDRLDE aa  1-16(16+2 aa)
peptide 2: biotin-GC-VKEQVAEVRAKLEEQA aa 17-32(16+2 aa)
peptide 3: biotin-GC-QQIRLQAEAFQARLKS aa 33-48(16+2 aa)
peptide 4: biotin-GC-WFEPLVEDMQRQWAGL aa 49-64(16+2 aa)
peptide 5: biotin-GC-VEKVQAAVGTSAAPVP aa 65-80(16+2 aa)

peptide 6: biotin-GC-RTRDRLDEVKEQVAEV aa  9-24(16+2 aa)
peptide 7: biotin-GC-RAKLEEQAQQIRLQAE aa 25-40(16+2 aa)
peptide 8: biotin-GC-AFQARLKSWFEPLVED aa 41-56(16+2 aa)
peptide 9: biotin-GC-MQRQWAGLVEKVQAAV aa 57-72(16+2 aa)
peptide 10:biotin-GC-GTSAAPVPSDNH     aa 73-84(12+2 aa)
```

Fig.16.

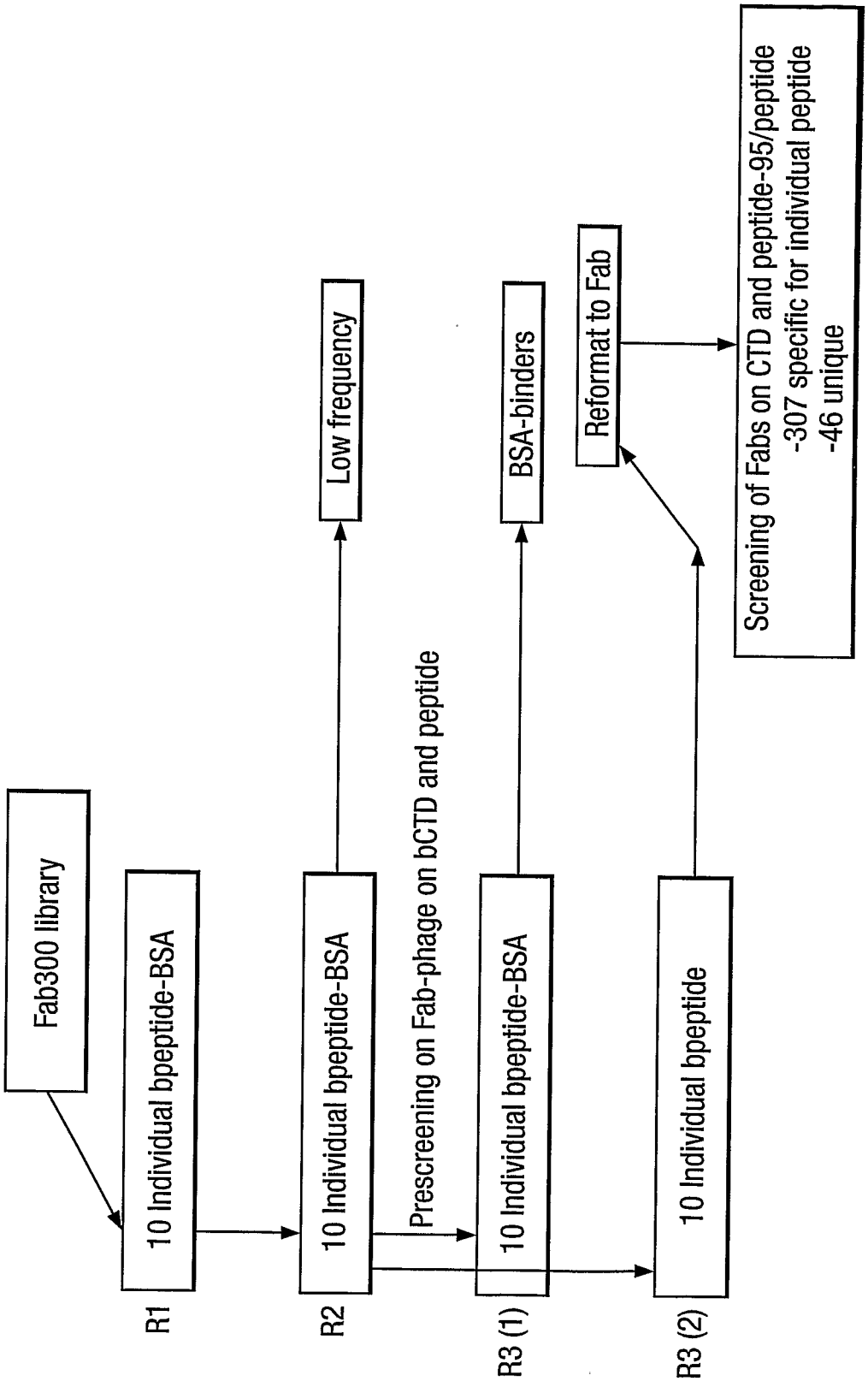

Fab300 library
10 Individual bpeptide-BSA
R1
10 Individual bpeptide-BSA
R2
Low frequency
Prescreening on Fab-phage on bCTD and peptide
10 Individual bpeptide-BSA
R3 (1)
BSA-binders
10 Individual bpeptide
R3 (2)
Reformat to Fab
Screening of Fabs on CTD and peptide-95/peptide
-307 specific for individual peptide
-46 unique

Fig.17.

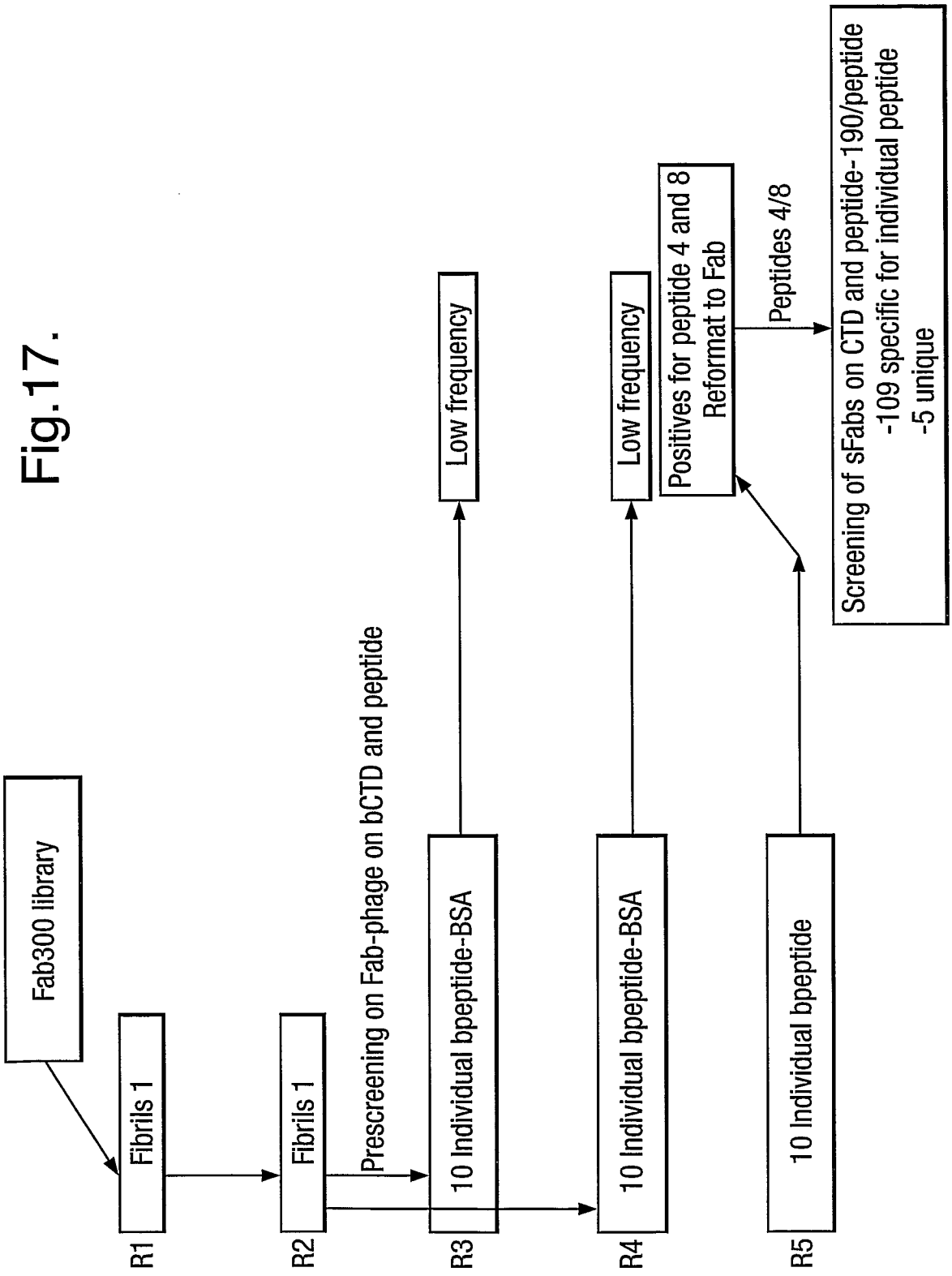

Fab300 library
Fibrils 1
R1
Fibrils 1
R2
Prescreening on Fab-phage on bCTD and peptide
10 Individual bpeptide-BSA
R3
Low frequency
10 Individual bpeptide-BSA
R4
Low frequency
Positives for peptide 4 and 8
Reformat to Fab
Peptides 4/8
10 Individual bpeptide
R5
Screening of sFabs on CTD and peptide-190/peptide
-109 specific for individual peptide
-5 unique

Fig.18.

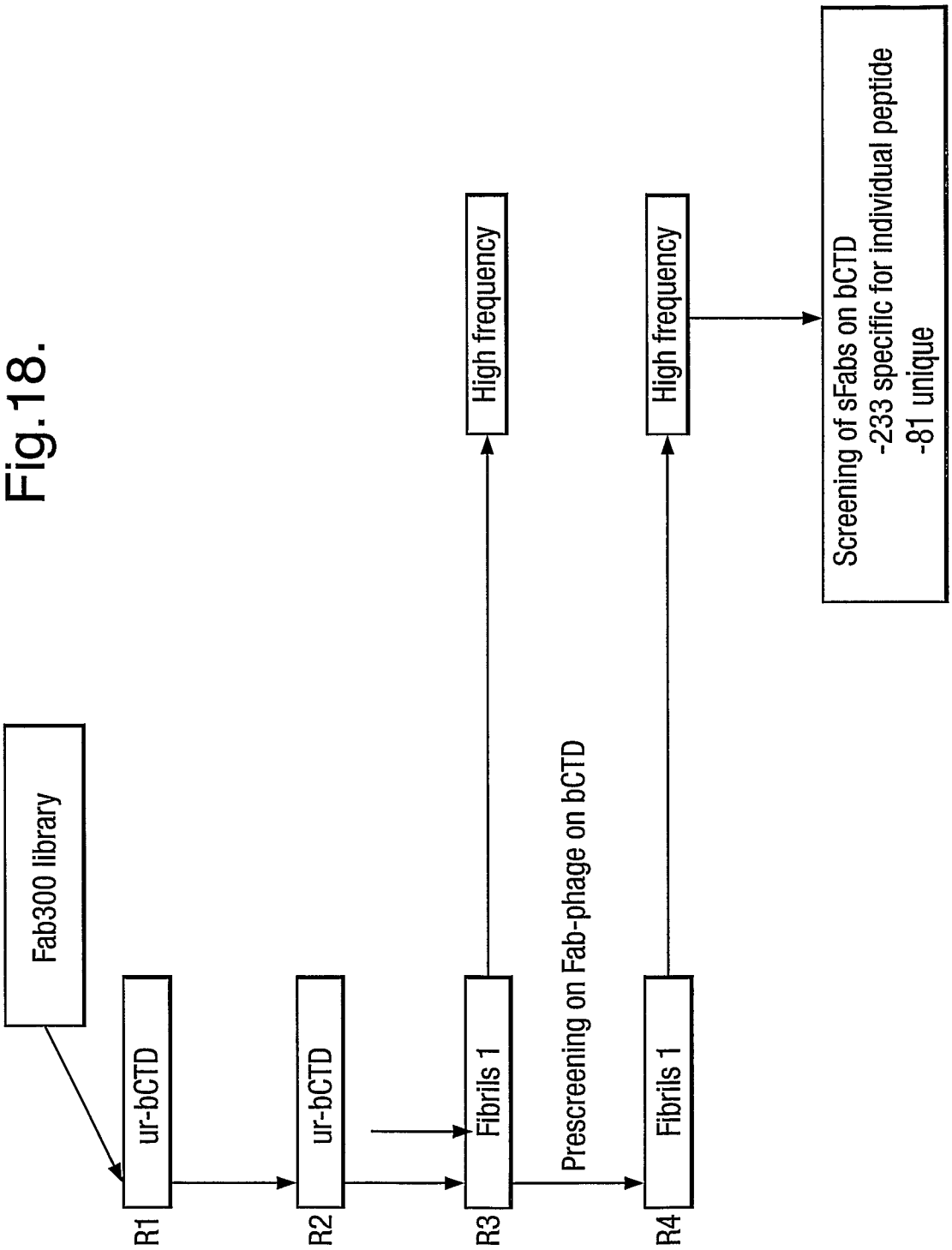

Fab300 library
ur-bCTD
ur-bCTD
Fibrils 1
Prescreening on Fab-phage on bCTD
Fibrils 1
High frequency
High frequency
Screening of sFabs on bCTD
-233 specific for individual peptide
-81 unique
R1
R2
R3
R4

Fig.19.

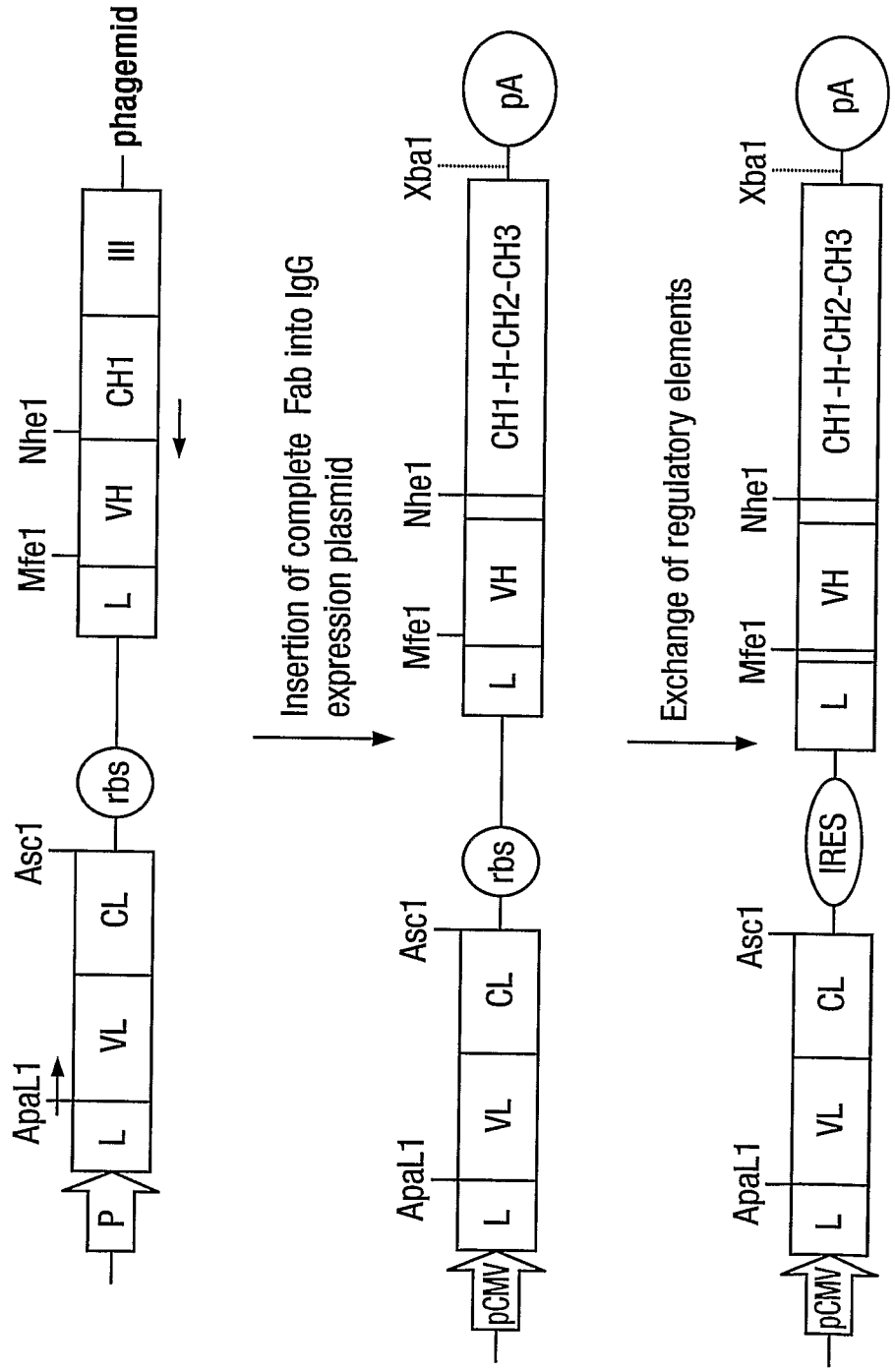

phagemid
III
CH1
VH
L
Nhe1
Mfe1
rbs
CL
VL
L
Asc1
ApaL1
P
Insertion of complete Fab into IgG expression plasmid
pA
Xba1
CH1-H-CH2-CH3
VH
L
Nhe1
Mfe1
rbs
CL
VL
L
Asc1
ApaL1
pCMV
Exchange of regulatory elements
pA
Xba1
CH1-H-CH2-CH3
VH
L
Nhe1
Mfe1
IRES
CL
VL
L
Asc1
ApaL1
pCMV

Fig.20.

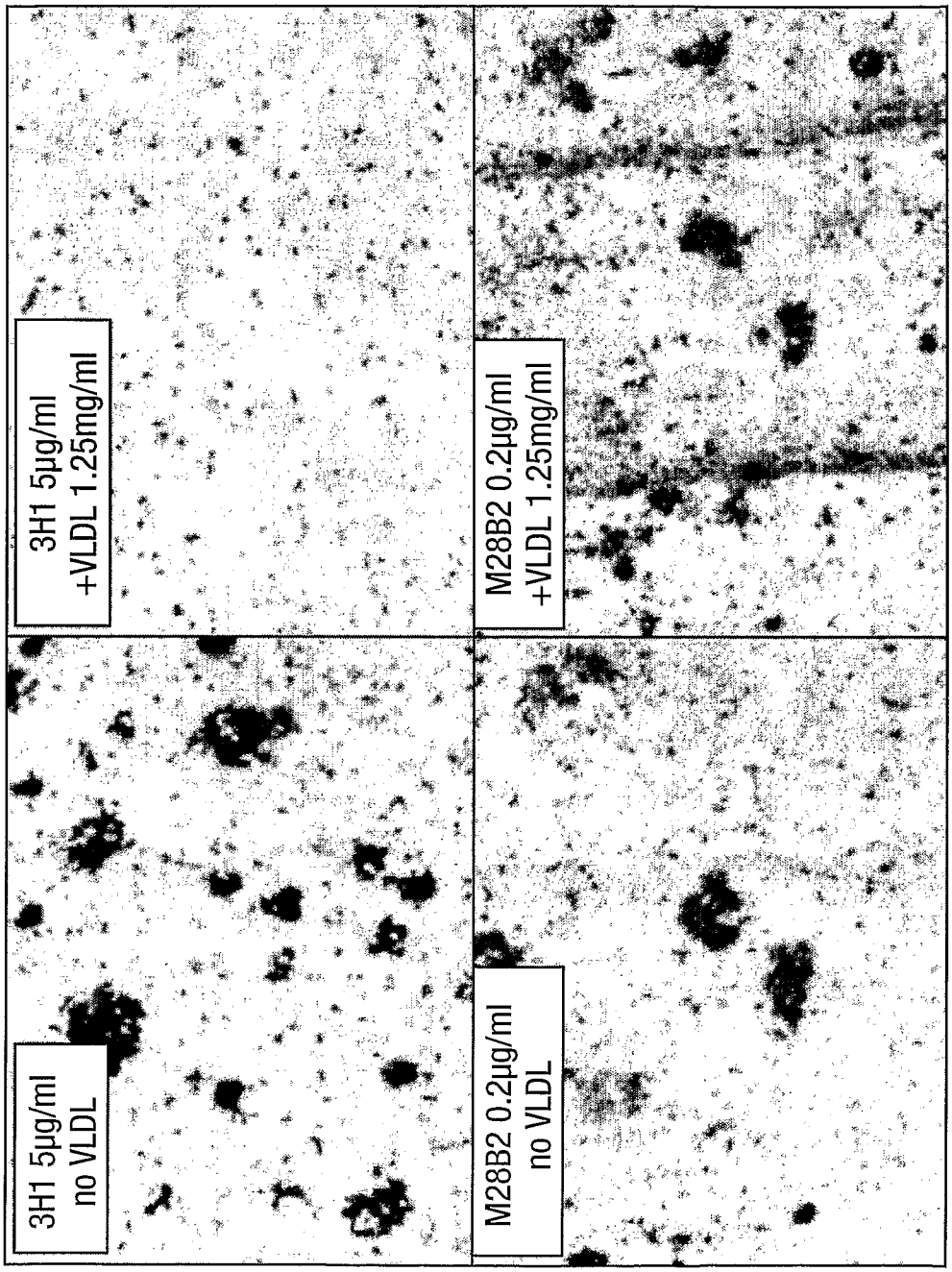

3H1 5µg/ml
no VLDL
3H1 5µg/ml
+VLDL 1.25mg/ml
M28B2 0.2µg/ml
no VLDL
M28B2 0.2µg/ml
+VLDL 1.25mg/ml

Fig.21.

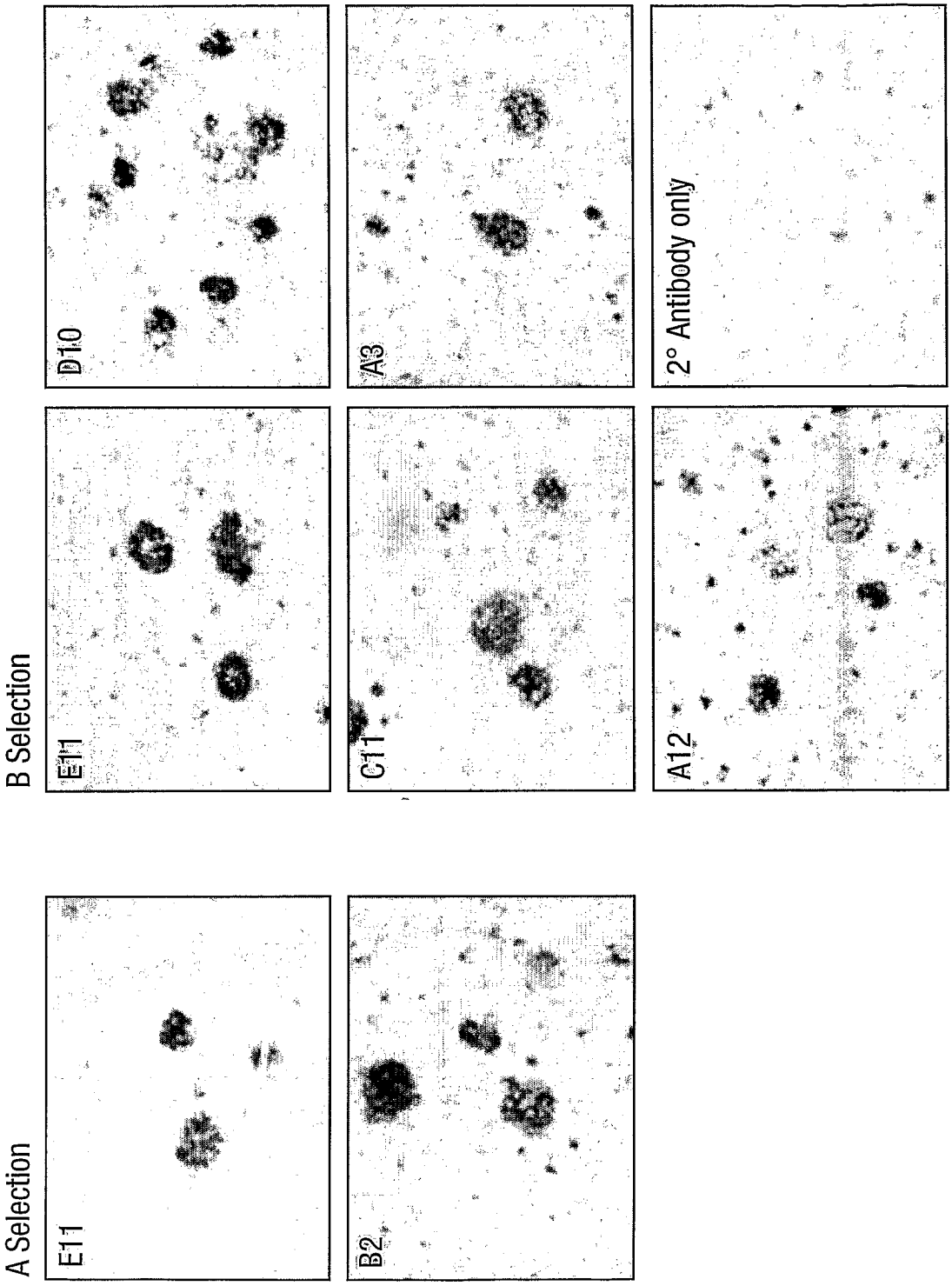

A Selection
E11
B2
B Selection
E11
C11
D10
A3
A12
2° Antibody only

Fig.22.

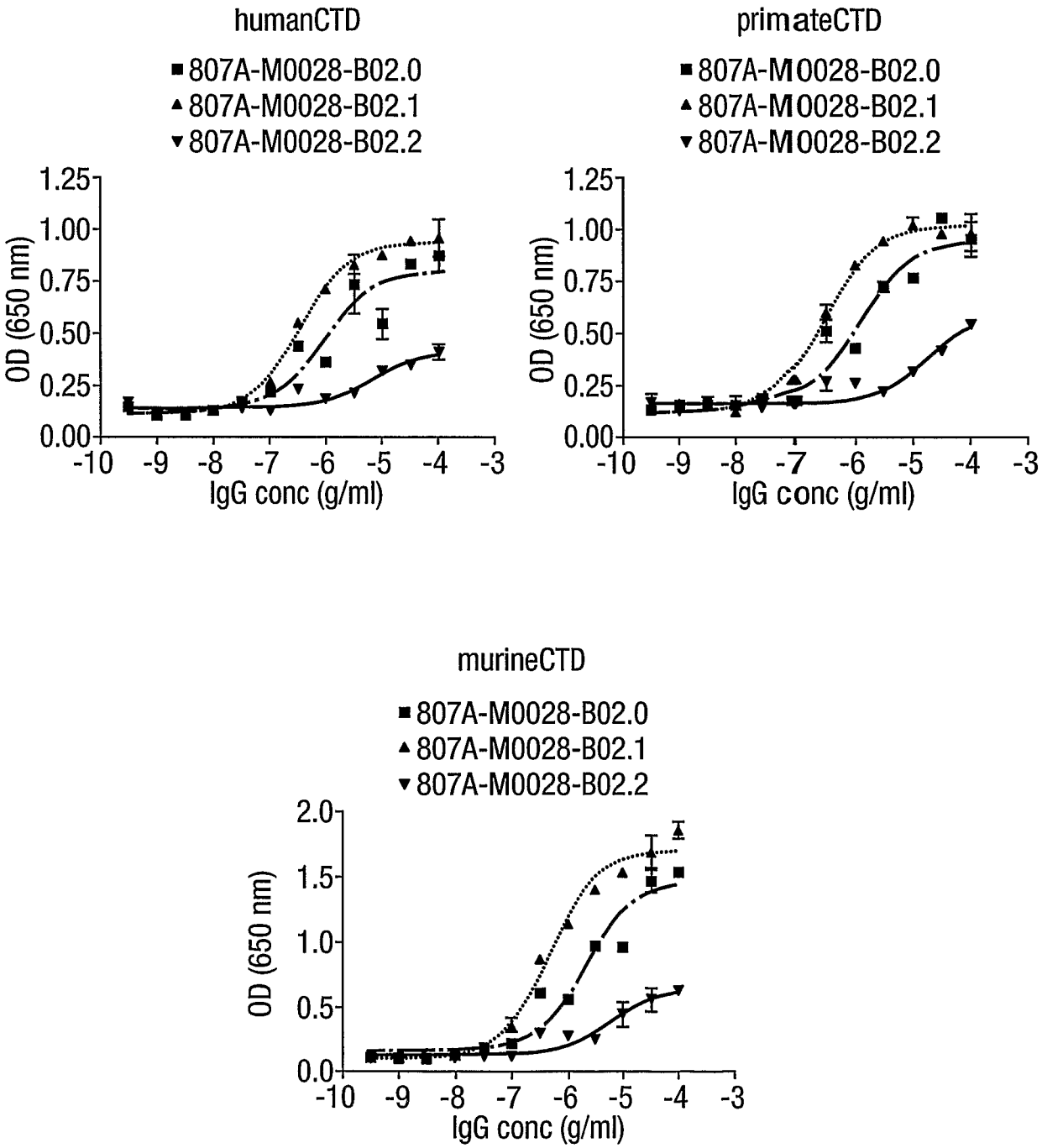

humanCTD
807A-M0028-B02.0
807A-M0028-B02.1
807A-M0028-B02.2
OD (650 nm)
1.25
1.00
0.75
0.50
0.25
0.00
-10 -9 -8 -7 -6 -5 -4 -3
IgG conc (g/ml)
primateCTD
807A-M0028-B02.0
807A-M0028-B02.1
807A-M0028-B02.2
OD (650 nm)
1.25
1.00
0.75
0.50
0.25
0.00
-10 -9 -8 -7 -6 -5 -4 -3
IgG conc (g/ml)
murineCTD
807A-M0028-B02.0
807A-M0028-B02.1
807A-M0028-B02.2
OD (650 nm)
2.0
1.5
1.0
0.5
0.0
-10 -9 -8 -7 -6 -5 -4 -3
IgG conc (g/ml)

Fig.23.

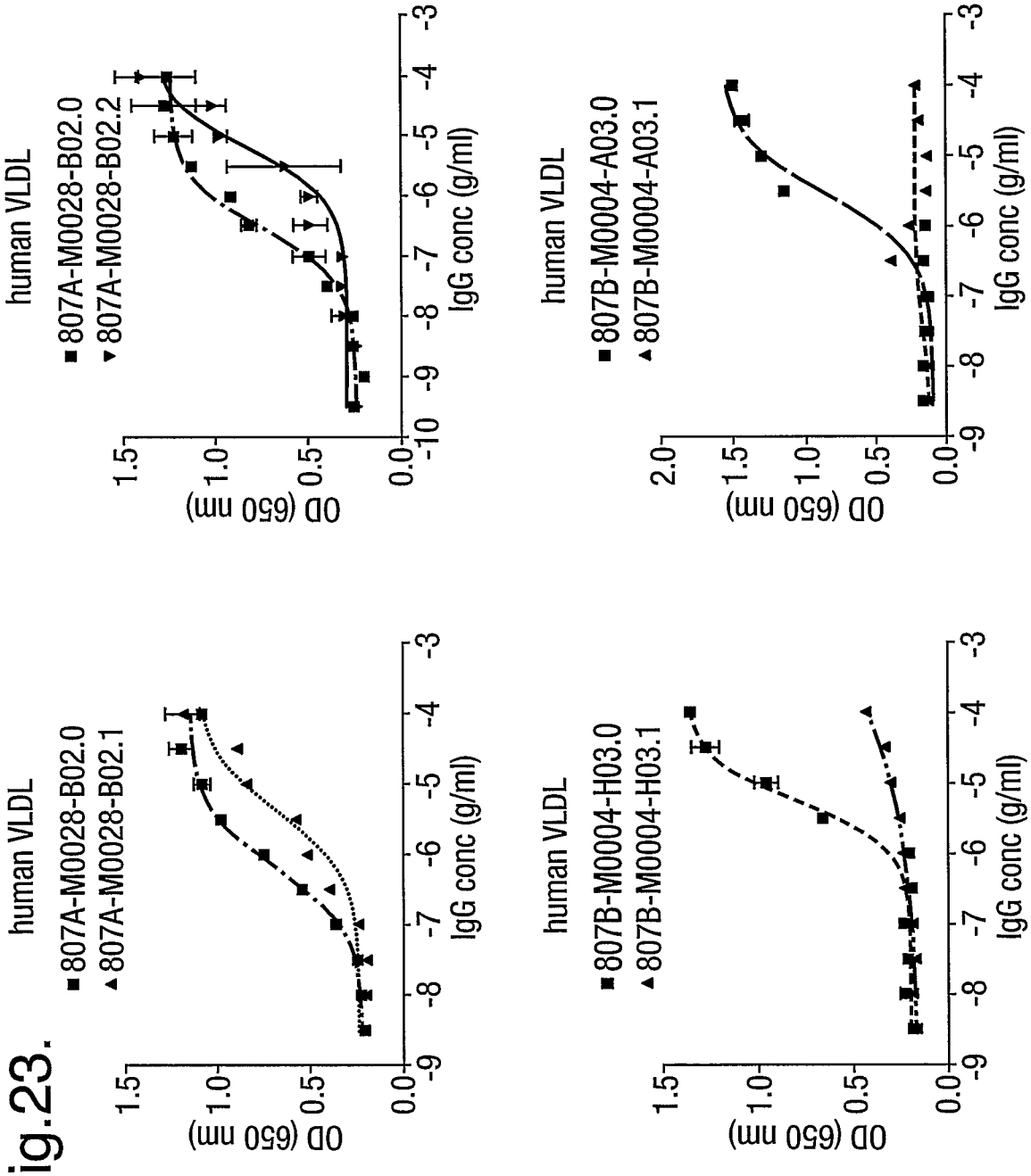

human VLDL
807A-M0028-B02.0
807A-M0028-B02.1
807A-M0028-B02.2
807B-M0004-A03.0
807B-M0004-A03.1
807B-M0004-H03.0
807B-M0004-H03.1
OD (650 nm)
IgG conc (g/ml)

Fig.24A.

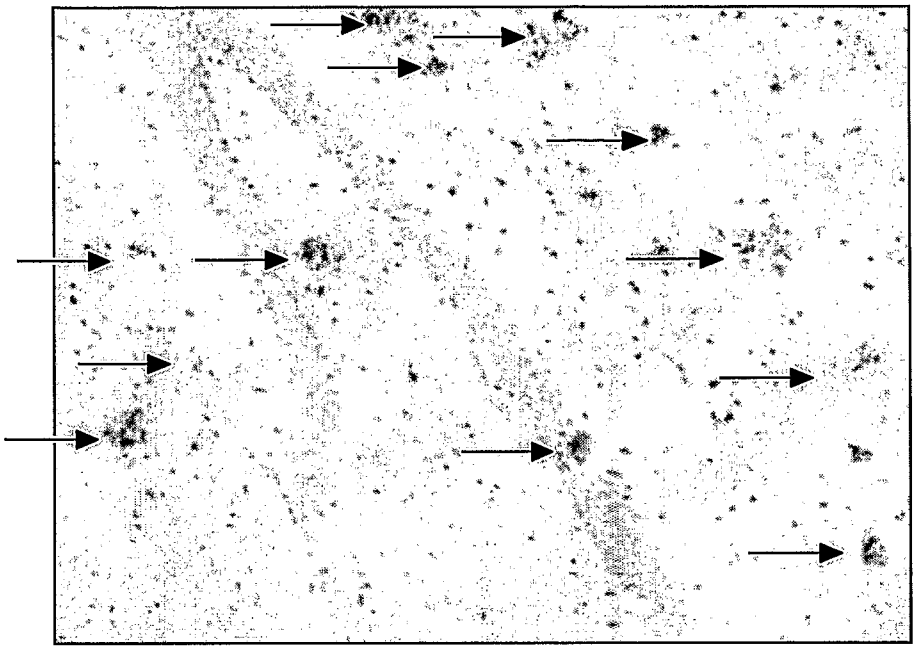


Fig.24B.

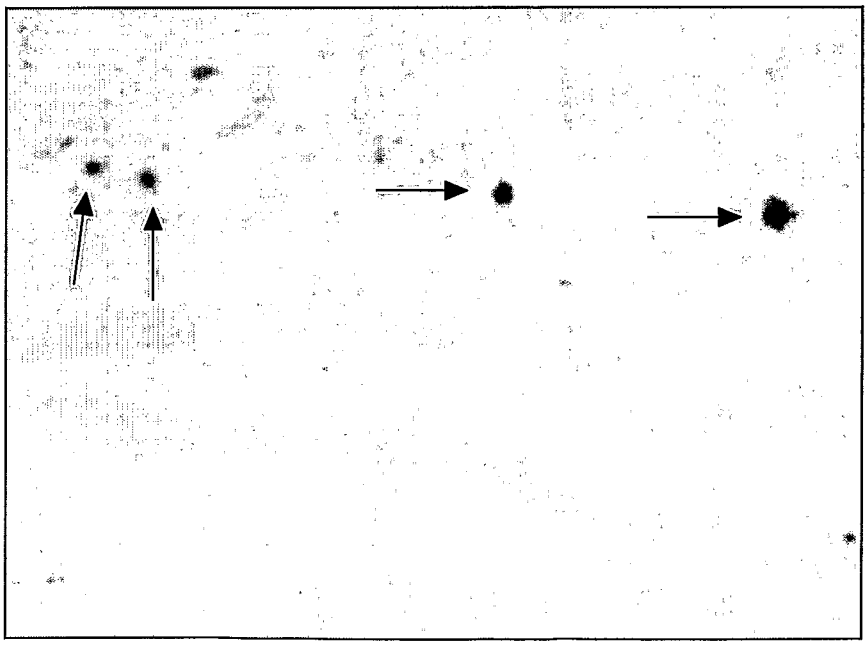


Fig.25.

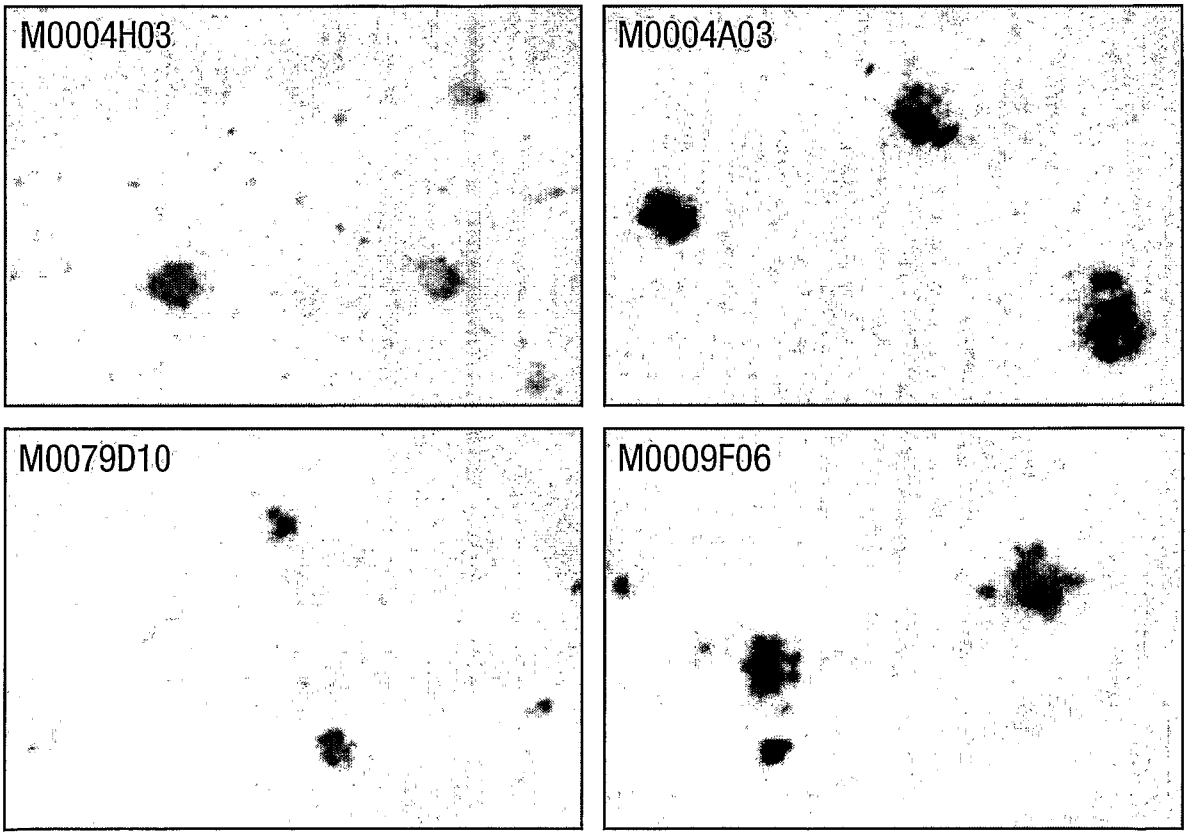


Fig.26.

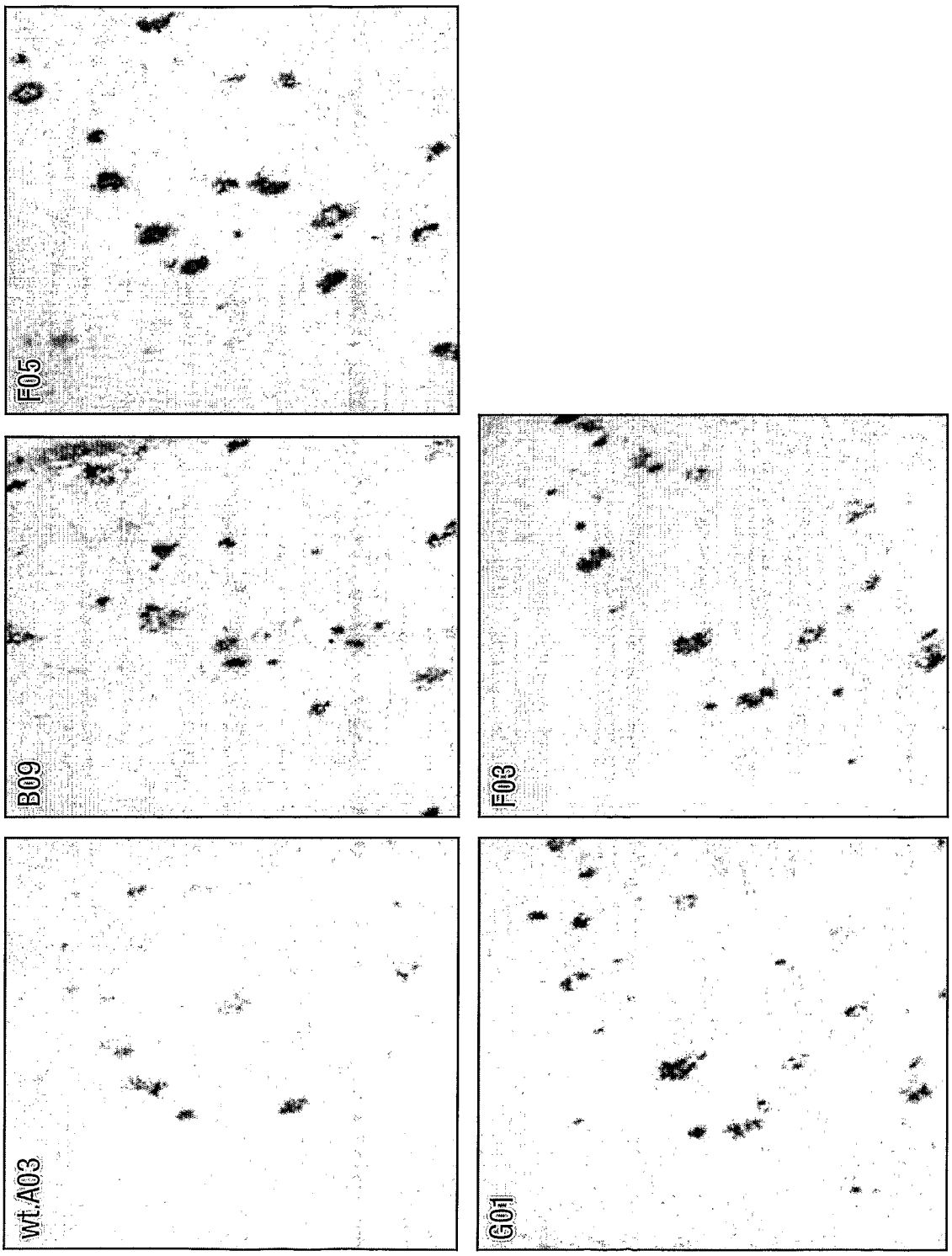
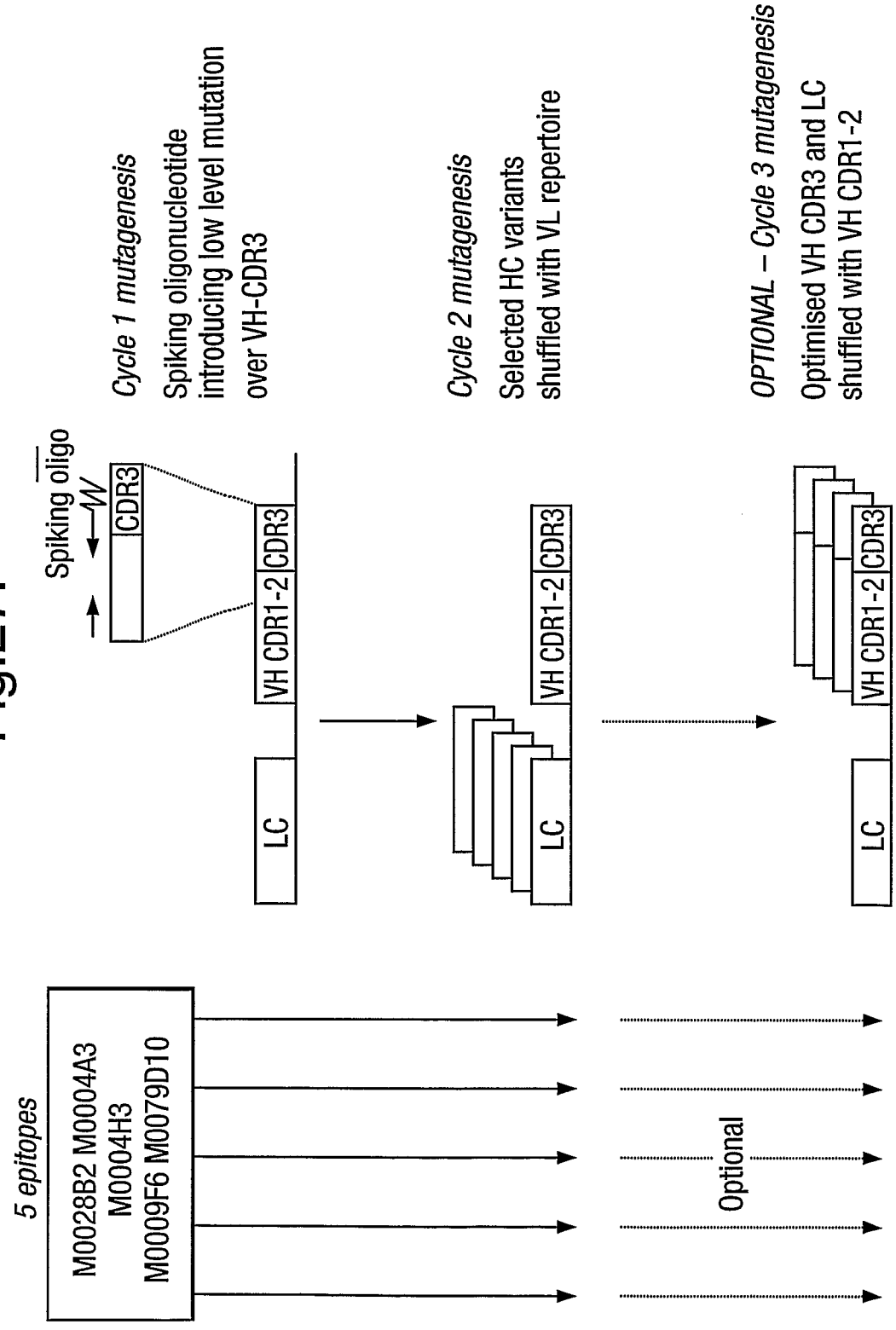


Fig.27.


5 epitopes
M0028B2 M0004A3
M0004H3
M0009F6 M0079D10
Optional
Cycle 1 mutagenesis
Spiking oligonucleotide introducing low level mutation over VH-CDR3
Spiking oligo
CDR3
LC
VH CDR1-2
CDR3
Cycle 2 mutagenesis
Selected HC variants shuffled with VL repertoire
OPTIONAL – Cycle 3 mutagenesis
Optimised VH CDR3 and LC shuffled with VH CDR1-2